09/121798
Aff#

WEST Search History

DATE: Thursday, July 18, 2002

Set Name side by side	Query	Hit Count	Set Name result set
DB=USPT,PGPB,JPAB,EPAB,DWPI; PLUR=YES; OP=ADJ			
L18	l1 with l6 with l15 and l2	29	L18
L17	l1 with l6 with l15	74	L17
L16	l1 with L15	793	L16
L15	large scale	111761	L15
L14	l1 with l6 and l2 and l3	200	L14
L13	l1 same l2 same l3 same l6	3	L13
L12	l1 same l2 same l6 same l6	154	L12
L11	l1 same l2 same l6	154	L11
L10	l1 with l2 with l6	13	L10
L9	L8 and l2 and l3	200	L9
L8	l1 with l6	2559	L8
L7	l1 with l2	153	L7
L6	lysis or lysing or lysed or lyse	35693	L6
L5	l1 with l2 and l3	45	L5
L4	l1 with l2	153	L4
L3	ion (exchange or exchanger)	111393	L3
L2	neutraliz$	167892	L2
L1	plasmid	48852	L1

END OF SEARCH HISTORY

09/121,788
AF/#24

=> s plasmid?
L1 334257 PLASMID?

=> s large scale
L2 123300 LARGE SCALE

=> s l1 and l2
L3 1806 L1 AND L2

=> s neutraliz?
L4 297568 NEUTRALIZ?

=> s l1 and l2 and l3

L5 1806 L1 AND L2 AND L3

=> s l1 and l2 and l4
L6 14 L1 AND L2 AND L4

=> dup rem l6
PROCESSING COMPLETED FOR L6
L7 10 DUP REM L6 (4 DUPLICATES REMOVED)

=> d l7 ibib abs 1-10

L7 ANSWER 1 OF 10 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2002:159961 BIOSIS
DOCUMENT NUMBER: PREV200200159961
TITLE: Insect cell production of a secreted form of human alpha1-proteinase inhibitor as a bifunctional protein which inhibits neutrophil elastase and has growth factor-like activities.
AUTHOR(S): Curtis, Heather; Sandoval, Carolyn; Oblin, Colette; Difalco, Marcos R.; Congote, L. Fernando (1)
CORPORATE SOURCE: (1) Departments of Experimental Medicine and Biochemistry, Endocrine Laboratory, McGill University Health Centre, 687 Avenue des Pins, Ouest, Montreal, PQ, H3A 1A1: luis.f.congote@muhc.mcgill.ca Canada
SOURCE: Journal of Biotechnology, (31 January, 2002) Vol. 93, No. 1, pp. 35-44. print. ISSN: 0168-1656.
DOCUMENT TYPE: Article
LANGUAGE: English
AB alpha1-proteinase inhibitor (API) is a potential therapeutic agent in all diseases in which elastase released by neutrophils has to be effectively ***neutralized*** . We ligated the cDNA of human API to the C-terminal section of an insulin-like growth factor II analogne (BOMIGF), known to be properly folded and secreted in insect cells using the baculovirus expression system. The BOMIGF-API chimera was recovered from the incubation medium of the infected cells. It shared the properties of both IGFs and API. It inhibited neutrophil elastase and formed SDS-stable complexes with the enzyme. The attachment of the large API protein to the C-terminal end of the 10 kDa IGF analogue did not destroy the IGF-mediated stimulation of thymidine incorporation into bovine fetal erythroid cells. We tested the capacity of the chimera to affect fibronectin-dependent TF-1 cell migration. BOMIGF-API significantly restored TF-1 cell migration in the presence of elastase, which is the enzyme of burn wound fluid most probably involved in fibronectin degradation. Some of the beneficial uses for this chimera may include all instances for which inhibition of elastase-mediated extracellular matrix destruction as well as stimulation of cell migration and proliferation are required for tissue repair.

L7 ANSWER 2 OF 10 WPIDS (C) 2002 THOMSON DERWENT
ACCESSION NUMBER: 2002-055343 [07] WPIDS
DOC. NO. CPI: C2002-015810
TITLE: Histone H2A-derived peptides useful in gene delivery and gene therapy.
DERWENT CLASS: B04 D16
INVENTOR(S): BALICKI, D; BEUTLER, E
PATENT ASSIGNEE(S): (NOVS) NOVARTIS AG; (NOVS) NOVARTIS-ERFINDUNGEN VERW GES MBH; (SCRI) SCRIPPS RES INST
COUNTRY COUNT: 95
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2001081370	A2	20011101	(200207)*	EN	36

RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW
AU 2001056319 A 20011107 (200219)

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2001081370	A2	WO 2001-EP4621	20010424
AU 2001056319	A	AU 2001-56319	20010424

FILING DETAILS:

PATENT NO	KIND	PATENT NO
AU 2001056319	A Based on	WO 200181370

PRIORITY APPLN. INFO: US 2000-199153P 20000424
AN 2002-055343 [07] WPIDS
AB WO 200181370 A UPAB: 20020130
NOVELTY - An isolated gene delivery facilitating peptide (I) comprising at least 7 amino acids (preferably 17 amino acids) derived from the N-terminal region of Histone H2A, and which exhibits transfection activity and nuclear localization activity, is new.
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:
(1) a complex (II) comprising (I) complexed with a nucleic acid;
(2) a solution (III) comprising (II) and a transfection enhancing medium;
(3) a method (IV) for producing (II) comprising mixing (I) with a nucleic acid in a transfection enhancing medium to form a peptide-nucleic acid complex;
(4) a method (V) of transfecting a cell, comprising administering (II) to the cell;
(5) a cell (VI) transfected via (V);
(6) a manufactured article (VII) comprising a packaging material containing (I) which is useful for delivering a nucleic acid to cell (the packaging material comprises a label which indicates that the peptide can be used for delivering a nucleic acid into a cell when a H2A-derived peptide-nucleic complex is formed); and
(7) a nucleic acid (VIII) encoding (I).
ACTIVITY - None specified.
MECHANISM OF ACTION - Gene delivery (claimed); gene therapy; antisense therapy.
COS-7 (African green monkey SV40-transformed kidney cells) in culture were overlaid with 75 micro l/well of the binary DNA-histone H2A-derived peptide complex comprising the ***plasmid*** pCMV beta (a beta -galactosidase reporter ***plasmid***).
A peptide corresponding to the first 36 amino acids of histone H2A was effective in delivering the ***plasmid*** into recipient cells. Subsequently, a 17-mer that represents amino acids 18-34 of H2A was active in DNA delivery transfecting less than 1% of COS-7 cells, compared to 5-10% for the 36-mer.
USE - (I) Is useful for delivering a nucleic acid to cell (claimed).
ADVANTAGE - The gene delivery enhancing peptide, derived from Histone H2A is complexed with a nucleic acid for efficient and stable delivery of the nucleic acid into a cell, ultimately to the nucleus. The peptide

mediated gene delivery is based on the principle that un- ***neutralized*** positive charges on the Histone are bound electro-statically both by the negatively charged phosphate backbone of DNA and that nuclear targeting signals in the Histones improve trafficking of the DNA into the nucleus for transcription.

This mode of delivery overcomes the limitations of current gene delivery approaches including viral and non-viral means, has minimum toxicity, with cellular access, intracellular trafficking and nuclear retention of ***plasmids*** .

The entire H2A sequence is not essential for mediating efficient delivery of the nucleic acids into cells as opposed to the prior art use of the full sequence. In addition, substitutions to the sequences have also been found to mediate efficient delivery, providing an improved delivery system on the original H2A protein.

(I) Has a transfection activity at least twice (preferably 3 times) that of background levels (measured as described in the specification) (claimed).

Furthermore, (I) has the potential advantages of ease of use, production, and mutagenesis, purity, homogeneity, ability to target nucleic acids to specific cell types, cost effective ***large*** - ***scale*** manufacture, modular attachment of targeting ligands, and the lack of limitation on the size or type of the nucleic acid that can be delivered.

Dwg.0/2

L7 ANSWER 3 OF 10 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2001:554590 HCAPLUS
DOCUMENT NUMBER: 135:151708
TITLE: Saccharomyces expressing Bifidobacterium lactate dehydrogenase gene for manufacture of lactic acid under acidic conditions
INVENTOR(S): Kuromiya, Shigeru; Matsuo, Yasuo; Saito, Satoshi; Yamaguchi, Ikuo; Saotome, Satoru
PATENT ASSIGNEE(S): Toyota Motor Corp., Japan
SOURCE: Jpn. Kokai Tokkyo Koho, 7 pp.
CODEN: JKXXAF
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 3
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 2001204464	A2	20010731	JP 2000-18826	20000127
WO 2001055363	A1	20010802	WO 2001-JP552	20010126

W: CN, ID, IN, US, VN
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR

PRIORITY APPLN. INFO.: JP 2000-18826 A 20000127
JP 2000-18953 A 20000127
JP 2000-318009 A 20001018

AB This invention provides a transgenic Saccharomyces cerevisiae expression Bifidobacterium longum lactate dehydrogenase gene which can produce lactic acid under pH 6.0. The method provides in this invention can be used in ***large*** ***scale*** fermn. of lactic acid without ***neutralization*** of growing medium.

L7 ANSWER 4 OF 10 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2001:112928 HCAPLUS
DOCUMENT NUMBER: 135:237237
TITLE: Purification of essentially RNA free ***plasmid*** DNA using a modified Escherichia coli host strain expressing ribonuclease A
AUTHOR(S): Cooke, G. D.; Cranenburgh, R. M.; Hanak, J. A. J.; Dunnill, P.; Thatcher, D. R.; Ward, J. M.
CORPORATE SOURCE: Advanced Centre For Biochemical Engineering, Department of Biochemical Engineering, University College London, London, Torrington Place, WC1E 7JE, UK
SOURCE: Journal of Biotechnology (2001), 85(3), 297-304
CODEN: JBITD4; ISSN: 0168-1656
PUBLISHER: Elsevier Science Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Regulatory agencies have stringent requirements for the ***large*** - ***scale*** prodn. of biotherapeutics. One of the difficulties assocd. with the manuf. of ***plasmid*** DNA for gene therapy is the removal of the host cell-related impurity RNA following cell lysis. We have constructed a modified Escherichia coli JM107 ***plasmid*** host (JMRNaseA), contg. a bovine pancreatic RNase (RNaseA) expression cassette, integrated into the host chromosome at the dif locus. The expressed RNaseA is translocated to the periplasm of the cell, and is released during primary ***plasmid*** extn. by alk. lysis. The RNaseA protein is stable throughout incubation at high pH (.apprx.12-12.5), and subsequently acts to hydrolyze host cell RNA present in the ***neutralized*** soln. following alk. lysis. Results with this strain harboring pUC18, and a 2.4 kb pUC18.DELTA.lacO, show that sufficient levels of RNase activity are produced to hydrolyze the bulk of the host RNA. This provides a suitable methodol. for the removal of RNA, while avoiding the addn. of exogenous animal sourced RNase and its assocd. regulatory requirements.
REFERENCE COUNT: 25 THERE ARE 25 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 5 OF 10 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 1
ACCESSION NUMBER: 1997:132596 BIOSIS
DOCUMENT NUMBER: PREV199799424409
TITLE: A specific antibody response to HCV E2 elicited in mice by intramuscular inoculation of ***plasmid*** DNA containing coding sequences for E2.
AUTHOR(S): Tedeschi, Valeria; Akatsuka, Toshitaka; Shih, James Wai-Kuo; Battegay, Manuel; Feinstone, Stephen M. (1)
CORPORATE SOURCE: (1) Div. Viral Products, CBER/FDA, 29 Lincoln Dr., Build. 29A, Room 1D14, HFM448, Bethesda, MD 20892 USA
SOURCE: Hepatology, (1997) Vol. 25, No. 2, pp. 459-462.
ISSN: 0270-9139.
DOCUMENT TYPE: Article
LANGUAGE: English
AB As the chimpanzee, the only reliable animal model for hepatitis C virus (HCV) infection, is impractical for early stage testing of HCV vaccine candidates, we have evaluated the immune response in mice to an experimental ***plasmid*** based HCV vaccine. We used this system because DNA vaccines can be rapidly constructed without the necessity of ***large*** ***scale*** protein production and purification. In this preliminary study we tested the immune response in mice to HCV envelope glycoprotein, E2, induced by a eukaryotic expression ***plasmid*** . Protein expression was monitored by immunofluorescence in transfected tissue culture cells. Each mouse was inoculated intramuscular with 100 mu-g ***plasmid*** DNA and some mice were boosted after 5 weeks. Among 12 BALB/C mice inoculated, 10 developed antibody to E2 by the second week. The antibody levels increased steadily before reaching a plateau in mice receiving the booster, but in the nonboosted mice the antibody declined over time. The serum from one mouse was tested against a series of overlapping peptides covering most of E2. This serum contained antibodies recognizing two distinct epitopes beginning at amino acid 57 and amino acid 113 but no antibody was directed against peptides representing the hypervariable region of E2, antibody to which is thought to be important in HCV ***neutralization*** . We have shown that the use of ***plasmid*** based vaccines can induce a specific immune response in mice against HCV antigens. This system should be useful as the first step in vaccine development.

L7 ANSWER 6 OF 10 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1995:610625 HCAPLUS
DOCUMENT NUMBER: 123:8040
TITLE: Extraction of polypeptide inclusion bodies from expression hosts with a two-phase aqueous system with solubilization and renaturation of the polypeptide
INVENTOR(S): Builder, Stuart; Hart, Roger; Lester, Philip; Ogez, John; Reifsnyder, David
PATENT ASSIGNEE(S): Genentech, Inc., USA
SOURCE: PCT Int. Appl., 69 pp.

CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9506059	A1	19950302	WO 1994-US9089	19940810
W: AU, CA, JP, US				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
US 5407810	A	19950418	US 1993-110663	19930820
CA 2167910	AA	19950302	CA 1994-2167910	19940810
AU 9475616	A1	19950321	AU 1994-75616	19940810
AU 673624	B2	19961114		
EP 714403	A1	19960605	EP 1994-925830	19940810
EP 714403	B1	19980610		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE				
JP 09501931	T2	19970225	JP 1994-507623	19940810
AT 167193	E	19980615	AT 1994-925830	19940810
ES 2119222	T3	19981001	ES 1994-925830	19940810
US 5723310	A	19980303	US 1995-385187	19950207
US 5695958	A	19971209	US 1995-446882	19950517

PRIORITY APPLN. INFO.: US 1993-110663 19930820
WO 1994-US9089 19940810
US 1994-318627 19941011
US 1995-385187 19950207

AB A method is described for isolating an exogenous polypeptide in a non-native conformation from cells, such as an aq. fermn. broth. The inclusion bodies are incubated in a soln. of a chaotropic agent contg., preferably, a reducing agent and with phase-forming species to form multiple aq. phases, with one of the phases being enriched in the polypeptide and depleted in the biomass solids and nucleic acids originating from the cells. The method results in two aq. phases, with the upper phase being enriched in the polypeptide. A ***large*** ***scale*** (1200 L) fermn. of Escherichia coli accumulating inclusion bodies of insulin-like growth factor 1 as a result of expression of the cloned gene was lysed with urea 174 kg and dithiothreitol 2.9 kg and brought to pH 10 with NaOH. The lysate was mixed with PEG-8000 250 and sodium sulfate 90 kg and the phases allowed to sep. The upper phase contained 88% of the total IGF-1 in the prepn. The upper phase was collected and ***neutralized*** to ppt. the IGF-1 and the pptd. material was resuspended in a folding medium of urea 10, NaCl 1 M, EtOH 19 vol%, glycine 20 mM, copper 0.5 .mu.M, DTT 1mM pH 10.5. Renaturation had reached a plateau at 3 h with a 50% yield of folded IGF-1.

L7 ANSWER 7 OF 10 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1995:373588 HCAPLUS
DOCUMENT NUMBER: 122:206417
TITLE: Expression of the rotavirus SA11 protein VP7 in the simple eukaryote Dictyostelium discoideum
AUTHOR(S): Emslie, Kerry R.; Miller, Janine M.; Slade, Martin B.; Dormitzer, Philip R.; Greenberg, Harry B.; Williams, Keith L.
CORPORATE SOURCE: School of Biological Sciences, Macquarie University, Sydney, 2109, Australia
SOURCE: J. Virol. (1995), 69(3), 1747-54
CODEN: JOVIAM; ISSN: 0022-538X
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The outer capsid protein of rotavirus, VP7, is a major ***neutralization*** antigen and is considered a necessary component of any subunit vaccine developed against rotavirus infection. For this reason, significant effort has been directed towards producing recombinant VP7 that maintains the antigenic characteristics of the native mol. A relatively new expression system, the simple eukaryote Dictyostelium discoideum, was used to clone the portion of simian rotavirus SA11 genome segment 9, encoding the mature VP7 protein, downstream of a native D. discoideum secretion signal sequence in a high-copy-no. extrachromosomal vector. The majority of the recombinant VP7 expressed by transformants was intracellular and was detected by Western immunoblot following gel electrophoresis as 2 or 3 bands with an apparent mol. mass of 35.5-37.5 kDa. A small amt. of VP7 having an apparent mol. mass of 37.5 kDa was secreted. Both the intracellular VP7 and the secreted VP7 were N glycosylated and sensitive to endoglycosidase H digestion. Under nonreducing electrophoresis conditions, over half the intracellular VP7 migrated as a monomer while the remainder migrated with an apparent mol. mass approx. twice that of the monomeric form. In an ELISA, intracellular VP7 reacted with both nonneutralizing and ***neutralizing*** antibodies. The monoclonal antibody recognition pattern paralleled that found with VP7 expressed in either vaccinia virus or herpes simplex virus type 1 and confirms that D. discoideum-expressed VP7 is able to form the major ***neutralization*** domains present on viral VP7. Because D. discoideum cells are easy and cheap to grow, this expression system provides a valuable alternative for the ***large*** - ***scale*** prodn. of recombinant VP7 protein.

L7 ANSWER 8 OF 10 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 91027434 EMBASE
DOCUMENT NUMBER: 1991027434
TITLE: Recombinant cholera toxin B subunit and gene fusion proteins for oral vaccination.
AUTHOR: Sanchez J.; Johansson S.; Lowenadler B.; Svennerholm A.M.; Holmgren J.
CORPORATE SOURCE: Oficina de Correos No 1, Appartado Postal No 222,C.P. 6200 Cuernavaca, Morelos, Mexico
SOURCE: Research in Microbiology, (1990) 141/7-8 (971-979).
ISSN: 0923-2508 CODEN: RMCREW
COUNTRY: France
DOCUMENT TYPE: Journal; Conference Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English

AB The B subunit portion of cholera toxin (CTB) is a safe and effective oral immunizing agent in humans, affording protection against both cholera and diarrhoea caused by enterotoxigenic Escherichia coli producing heat-labile toxin (LT) (Clemens et al., 1986; 1988). CTB may also be used as a carrier of various 'foreign' antigens suitable for oral administration. To facilitate ***large*** - ***scale*** production of CTB for vaccine development purposes, we have constructed recombinant overexpression systems for CTB proteins in which the CTB gene is under the control of strong foreign (non-cholera) promoters and in which it is also possible to fuse oligonucleotides to the CTB gene and thereby achieve overexpression of hybrid proteins (Sanchez and Holmgren, 1989; Sanchez et al., 1988). We here expand these findings by describing overexpression of CTB by a constitutive tacP promoter as well as by the T7 RNA-polymerase promoter, and also by describing gene fusions leading to overexpression of several hybrid proteins between heat-stable E. coli enterotoxin (STa)-related peptides to either the amino or carboxy ends of CTB. Each of the hybrid proteins, when tested as immunogens in rabbits, stimulated significant anti-STa as well as anti-CTB antibody formation, although the anti-STa antibody levels attained (c.a. 1-15 .mu.g/ml specific anti-STa immunoglobulin) were too low to give more than partial neutalization of STa intestinal challenge in baby mice. The hybrid proteins also had a near-native conformation, as apparent from their oligomeric nature and their strong reactivity with both a ***neutralizing*** antibody against the B subunit and a ***neutralizing*** monoclonal antibody (mAb) against STa. However, only hybrid protein presenting the STa peptide with a free carboxy end was able to also react with another available STa mAb. Our results suggest that even minor modifications of a given antigenic region may lead to complete epitope hiding and/or to its lack of antibody reactivity. Alternate positioning of such peptides in the carboxy end of the CTB protein was found to assist in antibody recognition and is proposed as a means to help exposure of some foreign epitopes by CTB fusion proteins. The results may be of significance for the development of recombinant oral vaccines based on gene fusions to CTB or to the closely related B subunit of LT.

L7 ANSWER 9 OF 10 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1982:177435 HCAPLUS
DOCUMENT NUMBER: 96:177435
TITLE: A procedure for the ***large*** - ***scale*** isolation of highly purified ***plasmid*** DNA using alkaline extraction and binding to glass powder
AUTHOR(S): Marko, M. A.; Chipperfield, R.; Birnboim, H. C.
CORPORATE SOURCE: Radiat. Biol. Branch, Chalk River Nucl. Lab., Chalk River, ON, K0J 1J0, Can.
SOURCE: Anal. Biochem. (1982), 121(2), 382-7
CODEN: ANBCA2; ISSN: 0003-2697
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A preparative procedure for obtaining highly purified ***plasmid*** DNA from bacterial cells is described. The method is adapted from the earlier procedure (Birnboim, H. C.; Doly, J., 1979), which gave partially purified ***plasmid*** in a form suitable for rapid screening of a large no. of samples. In the present method, all detectable RNA, chromosomal DNA, and protein are removed (after ***neutralization*** of the alk. ext. and centrifugation) without the use of enzymes, PhOH extn., dialysis, or equil. centrifugation. Binding of ***plasmid*** DNA to glass powder in the presence of 6M NaClO4 is used for the final purifn. step.

L7 ANSWER 10 OF 10 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 2
ACCESSION NUMBER: 1981:210705 BIOSIS
DOCUMENT NUMBER: BA71:80697
TITLE: RAPID PURIFICATION OF COVALENTLY CLOSED CIRCULAR DNA OF BACTERIAL ***PLASMIDS*** AND ANIMAL TUMOR VIRUSES.
AUTHOR(S): MCMASTER G K; SAMULSKI R J; STEIN J L; STEIN G S
CORPORATE SOURCE: DEP. BIOCHEM. MOL. BIOL., UNIV. FLA., GAINESVILLE, FLA. 32610.
SOURCE: ANAL BIOCHEM, (1980 (RECD 1981)) 109 (1), 47-54.
CODEN: ANBCA2. ISSN: 0003-2697.
FILE SEGMENT: BA; OLD
LANGUAGE: English
AB A rapid and simple purification of covalently closed circular (supercoiled) DNA from bacterial clones (***plasmids***) and African green monkey cells (SV40-infected) is presented. The method involves immediate treatment of lysed cells with NaOH, followed by ***neutralization*** and phenol extraction in high salt. After the extraction mixture is centrifuged, supercoiled DNA is found in the aqueous phase, the noncovalently closed DNA molecules form a white precipitate at the interphase and proteins pellet. Contaminating RNA is eliminated from the aqueous phase by RNase treatment and precipitation of the supercoiled DNA with polyethylene glycol. Residual polyethylene glycol is removed from the resuspended DNA by chloroform extraction. The purified supercoiled DNA is compatible with restriction enzymes, and is efficient at transforming both .chi. 1776 and HB101 bacterial hosts. Centrifugation in ethidium bromide-cesium chloride or sucrose gradients is not necessary. The method is virtually independent of the molecular size and gives good yields of supercoiled DNA. The technique is applicable to ***large*** - ***scale*** preparations and as a rapid screening procedure in which 20-30 samples can be easily purified within 5-6 h.

=> d his

(FILE 'HOME' ENTERED AT 09:09:53 ON 18 JUL 2002)

FILE 'BIOSIS, EMBASE, MEDLINE, WPIDS, HCAPLUS' ENTERED AT 09:10:04 ON 18 JUL 2002
L1 334257 S PLASMID?
L2 123300 S LARGE SCALE
L3 1806 S L1 AND L2
L4 297568 S NEUTRALIZ?
L5 1806 S L1 AND L2 AND L3
L6 14 S L1 AND L2 AND L4
L7 10 DUP REM L6 (4 DUPLICATES REMOVED)

=> s static mixer
L8 2763 STATIC MIXER

=> s l1 and l8
L9 8 L1 AND L8

=> dup rem l9
PROCESSING COMPLETED FOR L9
L10 7 DUP REM L9 (1 DUPLICATE REMOVED)

=> d l10 ibib abs 1-7

L10 ANSWER 1 OF 7 WPIDS (C) 2002 THOMSON DERWENT
ACCESSION NUMBER: 2001-602251 [68] WPIDS
DOC. NO. CPI: C2001-178322
TITLE: Non-naturally occurring gene therapy vector useful for gene therapy, comprises an inner shell having a core complex containing a nucleic acid and at least one complex forming reagent.
DERWENT CLASS: A96 B04 B05 D16
INVENTOR(S): CHENG, C; FREI, J; METT, H; PUTHUPPARAMPIL, S; STANEK, J; SUBRAMANIAN, K; TITMAS, R; WOODLE, M; YANG, J
PATENT ASSIGNEE(S): (NOVS) NOVARTIS AG; (NOVS) NOVARTIS-ERFINDUNGEN VERW GES MBH
COUNTRY COUNT: 94
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2001049324	A2	20010712	(200168)*	EN	178

RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW
AU 2001033669 A 20010716 (200169)

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2001049324	A2	WO 2000-EP13300	20001228
AU 2001033669	A	AU 2001-33669	20001228

FILING DETAILS:

PATENT NO	KIND	PATENT NO
AU 2001033669	A Based on	WO 200149324

PRIORITY APPLN. INFO: US 1999-475305 19991230
AN 2001-602251 [68] WPIDS
AB WO 200149324 A UPAB: 20011121
NOVELTY - A non-naturally occurring gene therapy vector, comprising an inner shell having a core complex (1) containing a nucleic acid and at least one complex forming reagent (2), is new.
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:
(1) forming a self assembling core complex by feeding a stream of a solution of a nucleic acid and a core complex-forming moiety into a ***static*** ***mixer*** , the streams are split into inner and outer helical streams that intersect at several different points causing turbulence and promoting mixing, that results in a physicochemical assembly interaction; and

(2) a compound having formula (I).
m = 3 or 4;
Y = -(CH2)n-, or -CH2-CH=CH-CH2- if R2 is -(CH2)3-NR4R5 and m is 3;
n = 3-16;
R2 = H, or lower alkyl, or -(CH2)3-NR4R5 is m is 3;
R3 = H, or alkyl, or -CH2-CH(-X')-OH if R2 is -(CH2)3-NR4R5 and m is 3;
X and X' = independently, H or alkyl; and
R, R1, R4 and R5 = independently, H or lower alkyl, where R, R1, R4 and R5 are not all H or methyl, if m is 3 and Y is -(CH2)3.
ACTIVITY - None given.
MECHANISM OF ACTION - Gene therapy.
No biological data is given.
USE - In gene therapy for nucleic acid delivery.
ADVANTAGE - The vectors are stable having an improved outer steric layer that provides enhanced target specificity, in vivo and colloidal stability. The vectors are relatively homogenous and comprises chemically defined species. The vectors demonstrate improved cell entry and intracellular trafficking, permitting enhanced nucleic acid therapeutic activity such as gene expression.
Dwg.0/30

L10 ANSWER 2 OF 7 WPIDS (C) 2002 THOMSON DERWENT
ACCESSION NUMBER: 2002-033736 [04] WPIDS
DOC. NO. CPI: C2002-009357
TITLE: Purifying ***plasmid*** DNA from cells using anion exchange chromatography and hydrophobic interaction chromatography.
DERWENT CLASS: B04 D16
INVENTOR(S): DURLAND, R; HAYES, R; NOCHUMSON, S; WELP, J; WU, K; YU-SPEIGHT, A
PATENT ASSIGNEE(S): (VALE-N) VALENTIS INC
COUNTRY COUNT: 1
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
US 2001034435	A1	20011025	(200204)*		14

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
US 2001034435	A1	Provisional US 1996-22157P	19960719
		Cont of US 1997-887673	19970703
		US 2001-774284	20010129

PRIORITY APPLN. INFO: US 1996-22157P 19960719; US 1997-887673 19970703; US 2001-774284 20010129
AN 2002-033736 [04] WPIDS
AB US2001034435 A UPAB: 20020117
NOVELTY - Isolating ***plasmid*** DNA (M2) comprising lysing the cells containing the DNA to form a lysate, treating the lysate with a high salt agent forming a treated solution, and purifying the treated solution to provide isolated ***plasmid*** DNA, is new.
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the following:
(1) isolating (M2) ***plasmid*** DNA comprising:
(i) lysing cells containing the ***plasmid*** DNA with a lysis agent to form a lysate; and
(ii) purifying the lysate with anion exchange chromatography using a step gradient to produce isolated ***plasmid*** DNA (the isolated ***plasmid*** DNA is enriched with at least 80% supercoiled ***plasmid*** DNA);
(2) isolating ***plasmid*** DNA (M3) comprising:
(i) lysing cells containing the ***plasmid*** DNA with a lysis agent to form a lysate; and
(ii) using hydrophobic interaction chromatography to purify the lysate to produce isolated ***plasmid*** DNA;
(3) a device for isolating ***plasmid*** DNA from cells containing the ***plasmid*** DNA, comprising:
(i) a device for providing fast cell resuspension in a semi-continuous mode;
(ii) a device for providing mixing and cell lysis in a continuous flow mode; and
(iii) a device for providing chilling and mixing to denature and precipitate chromosomal DNA, protein, and RNA;
(4) isolating (M4) ***plasmid*** DNA, comprising:
(i) fermenting cells containing the ***plasmid*** DNA, harvesting the cells, and washing the cells;
(ii) exposing the cells to an alkaline lysis and neutralization agent to form a lysate;
(iii) performing centrifugation and filtration on the lysate;
(iv) treating the lysate with RNase at about 37 degrees Celsius for about one hour;
(v) filtrating the lysate and diluting the lysate with 2 volumes of WFI;
(vi) passing the lysate through a Q Sepharose HP resin a DEAE 650-S resin, and a Phenyl 650-S resin; and
(vii) filtrating the eluate from step 6 to yield the final product of isolated ***plasmid*** DNA; and
(5) isolating ***plasmid*** DNA (M5), comprising:
(i) fermenting cells containing the ***plasmid*** DNA, harvesting the cells, and washing the cells;
(ii) exposing the cells in an alkaline lysis and neutralization agent to form a lysate;
(iii) performing centrifugation or filtration on the lysate and performing a 1.5 volume dilution with WFI on the lysate;
(iv) exposing the lysate to an anionic change resin;
(v) washing the nicked and/or relaxed circular ***plasmid*** , as well as residual RNA, off of the resin with about O.6M NaCl;
(vi) eluting the ***plasmid*** DNA off of the resin with about 1.9 M ammonium sulfite;
(vii) passing the eluate through a hydrophobic interaction chromatography resin; and
(viii) filtrating the eluate to yield a final product of isolated ***plasmid*** DNA.
USE - The methods are used for purifying plasmid DNA from cells.
ADVANTAGE - The process (I) does not involve the use of RNase. At least 100 milligrams of the isolated plasmid DNA (pharmaceutical-grade plasmid DNA suitable for administration to humans) is obtained in (M1), (M2) and (M3). The processes are used for isolating plasmid DNA from lysate of a cell containing the plasmid DNA.
Dwg.0/3

L10 ANSWER 3 OF 7 WPIDS (C) 2002 THOMSON DERWENT
DUPLICATE 1
ACCESSION NUMBER: 2000-171430 [15] WPIDS
DOC. NO. CPI: C2000-053452
TITLE: Purifying nucleic acids from bacterial cells using static mixers for lysing cells and precipitating debris, followed by centrifugation and ion exchange chromatography.
DERWENT CLASS: B04 D16 J04
INVENTOR(S): BRIDENBAUGH, R; BUSSEY, L; DANG, W
PATENT ASSIGNEE(S): (VALE-N) VALENTIS INC
COUNTRY COUNT: 87
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2000005358	A1	20000203	(200015)*	EN	35
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL					
OA PT SD SE SL SZ UG ZW					
W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB					
GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU					
LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR					
TT UA UG US UZ VN YU ZA ZW					
AU 9948638	A	20000214	(200029)		
EP 1098966	A1	20010516	(200128)	EN	
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT					
RO SE SI					

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2000005358	A1	WO 1999-US15280	19990707
AU 9948638	A	AU 1999-48638	19990707
EP 1098966	A1	EP 1999-932304	19990707
		WO 1999-US15280	19990707

FILING DETAILS:

PATENT NO	KIND	PATENT NO
AU 9948638	A Based on	WO 200005358
EP 1098966	A1 Based on	WO 200005358

PRIORITY APPLN. INFO: US 1998-121798 19980723
AN 2000-171430 [15] WPIDS
AB WO 200005358 A UPAB: 20000323
NOVELTY - Purifying ***plasmid*** DNA from bacterial cells comprises lysing cells with a ***static*** ***mixer*** , precipitating them and centrifuging to isolate the clarified solution containing the ***plasmid*** DNA. The clarified solution is neutralized and contacted with a positively charged ion exchange chromatography resin to obtain a purified ***plasmid*** DNA solution.

DETAILED DESCRIPTION - Purifying ***plasmid*** DNA from bacterial cell comprises:

(a) contacting the cells with a lysis solution;

(b) passing it through a first ***static*** ***mixer*** to obtain lysed cell solution;

(c) contacting the solution with a precipitation solution;

(d) passing it through a second ***static*** ***mixer*** to obtain a precipitation mixture;

(e) centrifuging the mixture to isolate the clarified solution containing ***plasmid*** DNA;

(f) neutralizing the solution; and

(g) contacting the solution with a positively charged ion exchange chromatography resin and eluting the ***plasmid*** DNA from the resin with saline or a continuous gradient to obtain a purified ***plasmid*** DNA.

USE - The method is useful for purifying ***plasmid*** DNA from bacterial cells (claimed). The purified nucleic acid is used for variety of application e.g. molecular biological applications such as cloning or gene expression, or for diagnostic applications using e.g. Polymerase chain reaction (PCR), reverse transcriptase (RT)-PCR, dendromer formation etc., or for therapeutic uses, e.g. in gene therapy.

ADVANTAGE - The method minimizes complex and expensive purification steps, but yields high quality DNA, and so is economical. The method is suitable for providing pharmaceutical grade ***plasmid*** DNA.

Dwg.0/3

L10 ANSWER 4 OF 7 WPIDS (C) 2002 THOMSON DERWENT
ACCESSION NUMBER: 2000-224150 [19] WPIDS
DOC. NO. CPI: C2000-068331
TITLE: In-line mixing of nucleic acid molecules with a formulating agent to produce a stabilized co-lyophilized complex used in gene therapy.
DERWENT CLASS: A96 B04 B07 D16
INVENTOR(S): BRUNO, M; LAWSON, L; LOGAN, M J; MUMPER, R; TAGLIAFERRI, J
PATENT ASSIGNEE(S): (VALE-N) VALENTIS INC
COUNTRY COUNT: 87
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2000009086	A2	20000224	(200019)*	EN	94

RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL
OA PT SD SE SL SZ UG ZW
W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB
GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU
LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR
TT UA UG US UZ VN YU ZA ZW
AU 9953459 A 20000306 (200030)
EP 1104309 A2 20010606 (200133) EN
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT
RO SE SI

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2000009086	A2	WO 1999-US18064	19990810
AU 9953459	A	AU 1999-53459	19990810
EP 1104309	A2	EP 1999-939113	19990810
		WO 1999-US18064	19990810

FILING DETAILS:

PATENT NO	KIND	PATENT NO
AU 9953459	A Based on	WO 200009086
EP 1104309	A2 Based on	WO 200009086

PRIORITY APPLN. INFO: US 1998-96572P 19980814
AN 2000-224150 [19] WPIDS
AB WO 200009086 A UPAB: 20000419
NOVELTY - An in-line mixer containing a liquid, comprises a confined flowing system and the liquid comprises isolated, enriched or purified nucleic acid molecules.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are made for the following:

(1) a method of making an in-line mixer of the novelty, comprising adding liquid comprising isolated, enriched or purified nucleic acid molecules to the in-line mixer;

(2) a co-lyophilized complex, comprising a nucleic acid molecule in a vector and a formulating agent that protects the nucleic acid against freezing and increases its transfection rate; and

(3) a method of making a co-lyophilized complex of (2), comprising combining a first liquid comprising the nucleic acid molecule in a vector and a second liquid comprising the formulating agent, in an in-line mixer;

(4) a method of using the complex which comprises rehydrating the complex;

(5) a method of treating or preventing a disorder, comprising administering the complex of (2) to an animal; and

(6) a homogeneous mixture, comprising several complexes of (2), each having a uniform size.

ACTIVITY - Cytostatic; immunosuppressive; antiinflammatory; antilipemic; hypertensive; hypotensive; virucide; tuberculostatic; antiHIV; protozoacide; vasotropic.

MECHANISM OF ACTION - Gene therapy.

USE - The apparatus and method is used for preparation of a single-vial lyophilized nucleic acid/formulating agent complex which is of use in gene therapy. The complex may be used in the treatment of a wide variety of diseases including cancers e.g. epithelial glandular cancer, adenoma, adenocarcinoma, squamous and transitional cancer including polyp, papilloma, squamous cell and transitional cell cancer, including tissue type positive, sarcoma and other (oma's), hematopoietic and lymphoreticular cancer, including lymphoma, leukemia and Hodgkin's disease, neural tissue cancer, including neuroma, sarcoma, neurofibroma and blastoma, mixed tissues of origin cancer, including teratoma and teratocarcinoma and other cancerous conditions including cancer of the adrenal gland, anus, bile duct, bladder, brain tumors, breast, cancers of unknown primary site, carcinoids of the GI tract, cervix, childhood cancers, colon and rectum, esophagus, gall bladder, head and neck, islet cell and other pancreatic carcinomas, Kaposi's sarcoma, kidney, leukemia, liver, lung, non-small cell and small cell, lymphoma, Aids associated lymphoma, melanoma, mesothelioma, metastatic cancer, multiple myeloma, ovary, ovarian germ cell tumors, pancreas, parathyroid, penis, pituitary, prostate, sarcomas of the bone and soft tissue, skin, small intestine, stomach, testis, thymus, thyroid, trophoblastic disease, uterus, edometrial carcinoma, uterus, uterine sarcomas, vagina and vulva; nerve or muscle damage or atrophy, growth disorders, neuropathies, muscular dystrophy, Duchenne's muscular dystrophy, myotrophic disorders,

neurotrophic disorders, hemophilias, pituitary dwarfism, alpha 1-antitrypsin deficiency, autoimmune and inflammatory diseases, hypercholesterolemia, hypotension, hypertension, viruses, tuberculosis, HIV, malaria, and peripheral vascular disease.

ADVANTAGE - The formulation is provided in a form with relatively small and uniform particle size, with protection against degradation and an increased ability to transfect cells.

DESCRIPTION OF DRAWING(S) - The drawing illustrates a preferred set-up for the in-line mixing apparatus, the two liquids are fed in to the inlets and are driven by a pump to the Y-connector. Once the liquids have been brought into contact they are run through a ***static*** ***mixer*** to produce a homogenous complex with particles of approximately uniform size.

Dwg.1/2

L10 ANSWER 5 OF 7 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:388267 HCAPLUS
DOCUMENT NUMBER: 131:29582
TITLE: Method for purifying ***plasmid*** dna and ***plasmid*** dna substantially free of genomic dna
INVENTOR(S): Mcneilly, David S.
PATENT ASSIGNEE(S): Genzyme Corporation, USA
SOURCE: PCT Int. Appl., 17 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9929832	A1	19990617	WO 1998-US25581	19981203
W: CA, JP				
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
US 6214586	B1	20010410	US 1997-986885	19971208
CA 2311600	AA	19990617	CA 1998-2311600	19981203
EP 1036159	A1	20000920	EP 1998-962872	19981203
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
JP 2001526023	T2	20011218	JP 2000-524405	19981203

PRIORITY APPLN. INFO.: US 1997-986885 A 19971208
WO 1998-US25581 W 19981203

AB A method is described for purifying ***plasmid*** DNA from a mixt. contg. ***plasmid*** DNA and genomic DNA. The invention specifically provides a method for purifying Escherichia coli ***plasmid*** DNA that is scaleable to kilogram quantities that comprises: (a) lysing the cells using a static mixes; (b) pptg. the bulk of contaminating cellular components using a ***static*** ***mixer*** to obtain a clarified lysate contg. the ***plasmid*** DNA; (c) concg. the lysate by tangential flow ultrafiltration using a 100,000 MW cutoff membrane; (d) diafiltering the concd. lysate; (e) pptg. from the diafiltered lysate both bacterial genomic DNA and RNA by the addn. of ammonium sulfate; (f) obtaining a supernatant contg. purified ***plasmid*** DNA; (g) treating the supernatant by reverse phase chromatog. to obtain an eluant contg. purified ***plasmid*** DNA and (h) treating the eluant using anion exchange chromatog. The lysing step can be performed on cells directly from a fermenter. The purified E. coli ***plasmid*** DNA contg. genomic DNA in an amt. less than 0.2% by wt. based on the ***plasmid*** DNA. The purified ***plasmid*** DNA is suitable for use in humans.

REFERENCE COUNT: 2 THERE ARE 2 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L10 ANSWER 6 OF 7 WPIDS (C) 2002 THOMSON DERWENT
ACCESSION NUMBER: 1999-023457 [02] WPIDS
DOC. NO. CPI: C1999-007106
TITLE: Method for lysing cells while avoiding the shearing of genomic DNA - comprises providing ***static*** ***mixer*** , and simultaneously flowing cell suspension fluid and lysis solution through mixer.
DERWENT CLASS: B04 D16
INVENTOR(S): CHRISTOPHER, C W; MCNEILLY, D S; WAN, N C
PATENT ASSIGNEE(S): (GENZ) GENZYME CORP
COUNTRY COUNT: 1
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
US 5837529	A	19981117 (199902)*	8		

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
US 5837529	A Cont of	US 1994-324455	19941017
		US 1996-632203	19960415

PRIORITY APPLN. INFO: US 1994-324455 19941017; US 1996-632203 19960415
AN 1999-023457 [02] WPIDS
AB US 5837529 A UPAB: 19990113
Method for lysing cells while avoiding shearing genomic DNA, comprises providing a mixer and flowing a cell suspension fluid and a cell lysing solution through the mixer. the contact of the two liquids lyses the cells.

Also claimed is separating ***plasmids*** from ***plasmid*** containing cells using the method described above.

ADVANTAGE - The method is effective, economical and automatable.

Dwg.1/3

L10 ANSWER 7 OF 7 WPIDS (C) 2002 THOMSON DERWENT
ACCESSION NUMBER: 1997-351044 [32] WPIDS
DOC. NO. CPI: C1997-113445
TITLE: Lysing cells using static mixers - for preparation of DNAs as therapeutic agents for e.g. gene therapy.
DERWENT CLASS: B04 D16
INVENTOR(S): CHRISTOPHER, C W; MCNEILLY, D S; WAN, N C
PATENT ASSIGNEE(S): (GENZ) GENZYME CORP
COUNTRY COUNT: 20
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 9723601	A1	19970703 (199732)*	EN	17	
RW: AT BE CH DE DK ES FR GB GR IE IT LU MC NL PT SE					
W: AU CA JP					
AU 9646077	A	19970717 (199745)			
EP 811055	A1	19971210 (199803)	EN		
R: AT BE CH DE DK ES FR GB GR IE IT LI LU MC NL PT SE					
JP 11500927	W	19990126 (199914)		16	
AU 706857	B	19990624 (199936)			

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 9723601	A1	WO 1995-US16843	19951221
AU 9646077	A	WO 1995-US16843	19951221
		AU 1996-46077	19951221
EP 811055	A1	EP 1995-944222	19951221
		WO 1995-US16843	19951221
JP 11500927	W	WO 1995-US16843	19951221
		JP 1997-523593	19951221
AU 706857	B	WO 1995-US16843	19951221
		AU 1996-46077	19951221

FILING DETAILS:

PATENT NO	KIND	PATENT NO
AU 9646077	A Based on	WO 9723601
EP 811055	A1 Based on	WO 9723601
JP 11500927	W Based on	WO 9723601
AU 706857	B Previous Publ.	AU 9646077

Based on WO 9723601

PRIORITY APPLN. INFO: WO 1995-US16843 19951221
AN 1997-351044 [32] WPIDS
AB WO 9723601 A UPAB: 19970806
Lysing cells comprises simultaneously flowing a cell suspension and a lysis solution through a ***static*** ***mixer*** , where the cells exit the ***static*** ***mixer*** lysed. Also claimed are: (a) a method of precipitating cellular components from a solution, which comprises simultaneously flowing a cell lysate and a precipitating solution through a ***static*** ***mixer*** , where the cellular components exit the mixer precipitated, and (b) a method of releasing ***plasmids*** from cells, which comprises simultaneously flowing a suspension containing the cells and a lysis solution through a ***static*** ***mixer*** , where the cells exit the mixer lysed and ***plasmids*** released from the cells.
USE - The method can be used in the preparation of DNAs as therapeutic agents, i.e. in gene therapy, for the treatment of genetic diseases and for genetic immunisation.
ADVANTAGE - The method can be used for the treatment of multi-litre
amounts of solution containing multi-gram amounts of cells. These can be lysed rapidly, making large scale biological procedures involving cell lysis feasible.
Dwg.0/3

=> d his

(FILE 'HOME' ENTERED AT 09:09:53 ON 18 JUL 2002)

FILE 'BIOSIS, EMBASE, MEDLINE, WPIDS, HCAPLUS' ENTERED AT 09:10:04 ON 18
JUL 2002
L1 334257 S PLASMID?
L2 123300 S LARGE SCALE
L3 1806 S L1 AND L2
L4 297568 S NEUTRALIZ?
L5 1806 S L1 AND L2 AND L3
L6 14 S L1 AND L2 AND L4
L7 10 DUP REM L6 (4 DUPLICATES REMOVED)
L8 2763 S STATIC MIXER
L9 8 S L1 AND L8
L10 7 DUP REM L9 (1 DUPLICATE REMOVED)

=> s neutraliz?
L11 297568 NEUTRALIZ?

=> s ion(w)(exchange or exchanger)
L12 244833 ION(W)(EXCHANGE OR EXCHANGER)

=> s l1 and l11 and l12

L13 9 L1 AND L11 AND L12

=> dup rem l9
PROCESSING COMPLETED FOR L9
L14 7 DUP REM L9 (1 DUPLICATE REMOVED)

=> dup rem l13
PROCESSING COMPLETED FOR L13
L15 8 DUP REM L13 (1 DUPLICATE REMOVED)

=> d l15 ibib abs 1-8

L15 ANSWER 1 OF 8 WPIDS (C) 2002 THOMSON DERWENT
DUPLICATE 1
ACCESSION NUMBER: 2000-171430 [15] WPIDS
DOC. NO. CPI: C2000-053452
TITLE: Purifying nucleic acids from bacterial cells using static mixers for lysing cells and precipitating debris, followed by centrifugation and ***ion*** ***exchange*** chromatography.
DERWENT CLASS: B04 D16 J04
INVENTOR(S): BRIDENBAUGH, R; BUSSEY, L; DANG, W
PATENT ASSIGNEE(S): (VALE-N) VALENTIS INC
COUNTRY COUNT: 87
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2000005358	A1	20000203	(200015)*	EN	35

RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL
OA PT SD SE SL SZ UG ZW
W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB
GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU
LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR
TT UA UG US UZ VN YU ZA ZW
AU 9948638 A 20000214 (200029)
EP 1098966 A1 20010516 (200128) EN
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT
RO SE SI

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2000005358	A1	WO 1999-US15280	19990707
AU 9948638	A	AU 1999-48638	19990707
EP 1098966	A1	EP 1999-932304	19990707
		WO 1999-US15280	19990707

FILING DETAILS:

PATENT NO	KIND	PATENT NO
AU 9948638	A Based on	WO 200005358
EP 1098966	A1 Based on	WO 200005358

PRIORITY APPLN. INFO: US 1998-121798 19980723
AN 2000-171430 [15] WPIDS
AB WO 200005358 A UPAB: 20000323
NOVELTY - Purifying ***plasmid*** DNA from bacterial cells comprises
lysing cells with a static mixer, precipitating them and centrifuging to isolate the clarified solution containing the ***plasmid*** DNA. The clarified solution is ***neutralized*** and contacted with a positively charged ***ion*** ***exchange*** chromatography resin to obtain a purified ***plasmid*** DNA solution.
DETAILED DESCRIPTION - Purifying ***plasmid*** DNA from bacterial cell comprises:
(a) contacting the cells with a lysis solution;
(b) passing it through a first static mixer to obtain lysed cell solution;
(c) contacting the solution with a precipitation solution;
(d) passing it through a second static mixer to obtain a precipitation mixture;
(e) centrifuging the mixture to isolate the clarified solution containing ***plasmid*** DNA;
(f) ***neutralizing*** the solution; and
(g) contacting the solution with a positively charged ***ion*** ***exchange*** chromatography resin and eluting the ***plasmid*** DNA from the resin with saline or a continuous gradient to obtain a purified ***plasmid*** DNA.
USE - The method is useful for purifying ***plasmid*** DNA from bacterial cells (claimed). The purified nucleic acid is used for variety of application e.g. molecular biological applications such as cloning or gene expression, or for diagnostic applications using e.g. Polymerase chain reaction (PCR), reverse transcriptase (RT)-PCR, dendromer formation
etc., or for therapeutic uses, e.g. in gene therapy.
ADVANTAGE - The method minimizes complex and expensive purification
steps, but yields high quality DNA, and so is economical. The method is suitable for providing pharmaceutical grade ***plasmid*** DNA.
Dwg.0/3

L15 ANSWER 2 OF 8 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 95042862 EMBASE
DOCUMENT NUMBER: 1995042862

TITLE: Gel electrophoresis measurement of counterion condensation on DNA.
AUTHOR: Ma C.; Bloomfield V.A.
CORPORATE SOURCE: Department of Biochemistry, University of Minnesota,St. Paul, MN 55108, United States
SOURCE: Biopolymers, (1995) 35/2 (211-216). ISSN: 0006-3525 CODEN: BIPMAA
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
AB We used agarose gel electrophoresis to measure the effective charge ***neutralization*** of DNA by counterions of different structure and valence, including Na+, Mg2+, Co(NH3)6/3+, and spermidine3, which competed for binding with an excess of Tris acetate buffer. Linear DNA molecules ranged in size from 1 to 5 kilobases, and supercoiled ***plasmid*** pUC18 was also measured. In all cases, the results were in good agreement with theoretical predictions from counterion condensation theory for two-counterion mixtures.

L15 ANSWER 3 OF 8 MEDLINE
ACCESSION NUMBER: 95034710 MEDLINE
DOCUMENT NUMBER: 95034710 PubMed ID: 7947690
TITLE: Mutations affecting the activity of toxic shock syndrome toxin-1.
AUTHOR: Deresiewicz R L; Woo J; Chan M; Finberg R W; Kasper D L
CORPORATE SOURCE: Channing Laboratory, Boston, Massachusetts 02115.
SOURCE: BIOCHEMISTRY, (1994 Nov 1) 33 (43) 12844-51. Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199412
ENTRY DATE: Entered STN: 19950110
Last Updated on STN: 19950110
Entered Medline: 19941201
AB Toxic shock syndrome toxin-1 (TSST-1), the potent staphylococcal exoprotein linked to most cases of the toxic shock syndrome, is a V beta-restricted T-cell mitogen (a so-called "superantigen"). TSST-ovine (TSST-O) is a natural variant of TSST-1, and is produced by certain ovine mastitis-associated strains of Staphylococcus aureus. Compared to TSST-1, TSST-O is only weakly mitogenic for leporine or murine splenocytes. It differs from TSST-1 at 7 amino acid residues over its 194 amino acid length. Terminus shuffling between the two proteins has suggested that their C-terminal differences (T69, Y80, E132, and I140 in TSST-1; 169, W80, K132, and T140 in TSST-O) are in part responsible for their discrepant mitogenic properties. In order to explore further the functional consequences of altering TSST-1 at residues 132 and 140, we engineered point mutants of TSST-1 at those positions. The mutant proteins were purified to homogeneity from culture supernants of a nontoxigenic strain of S. aureus using a combination of ultrafiltration, liquid-phase isoelectric focusing, and ***ion*** - ***exchange*** chromatography. The mutants retained global structural integrity as evidenced by circular dichroism spectroscopy, their preserved resistance to trypsin digestion, and their preserved binding to a ***neutralizing*** murine monoclonal antibody. The mutants were then tested for mitogenicity for human T-cells: The mutant I140T was approximately as active as wild-type TSST-1, while the mutant E132D was about 10-fold attenuated. On the other hand, the mutants E132A or E132K were each at least 1000-fold attenuated.(ABSTRACT TRUNCATED AT 250 WORDS)

L15 ANSWER 4 OF 8 MEDLINE
ACCESSION NUMBER: 94153512 MEDLINE
DOCUMENT NUMBER: 94153512 PubMed ID: 7764434
TITLE: High yield production and purification of recombinant staphylokinase for thrombolytic therapy.
AUTHOR: Schlott B; Hartmann M; Guhrs K H; Birch-Hirschfeid E; Pohl H D; Vanderschueren S; Van de Werf F; Michoel A; Collen D; Behnke D
CORPORATE SOURCE: Institute for Molecular Biotechnology, Jena, Germany.
SOURCE: BIO/TECHNOLOGY, (1994 Feb) 12 (2) 185-9. Journal code: 8309273. ISSN: 0733-222X.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Biotechnology
ENTRY MONTH: 199403
ENTRY DATE: Entered STN: 19950809
Last Updated on STN: 20000303
Entered Medline: 19940331
AB Recombinant ***plasmids*** were constructed in which the signal sequence of the sak42D and the sakSTAR staphylokinase genes were replaced by an ATG start codon and which express staphylokinase under the control of a tac promoter and two Shine-Dalgarno sequences in tandem. Induction of transfected E. coli TG1 cells in a bacterial fermentor produced intracellular staphylokinase representing 10 to 15% of total cell protein. Gram quantities of highly purified recombinant staphylokinase were obtained from cytosol fractions by chromatography, at room temperature, on SP-Sepharose and on phenyl-Sepharose columns, with yields of 50 to 70 percent. The material, at a dose of 4 mg/kg, did not produce acute reactions or affect body weight in mice. Intravenous administration of 10 mg SakSTAR over 30 minutes in five patients with acute myocardial infarction induced complete coronary artery recanalization, without associated fibrinogen degradation. However, ***neutralizing*** antibodies appeared in the plasma of all patients within 12 to 20 days. Thus, the present expression and purification method for recombinant staphylokinase yields large amounts of highly purified mature protein (approximately 200 mg per liter fermentation broth) suitable for a more detailed clinical investigation of its potential as a thrombolytic agent.

L15 ANSWER 5 OF 8 MEDLINE
ACCESSION NUMBER: 93123218 MEDLINE
DOCUMENT NUMBER: 93123218 PubMed ID: 8380404
TITLE: Expression of the potato tuber ADP-glucose pyrophosphorylase in Escherichia coli.
AUTHOR: Iglesias A A; Barry G F; Meyer C; Bloksberg L; Nakata P A; Greene T; Laughlin M J; Okita T W; Kishore G M; Preiss J
CORPORATE SOURCE: Department of Biochemistry, Michigan State University, East Lansing 48824.
CONTRACT NUMBER: AI 022385 (NIAID)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1993 Jan 15) 268 (2) 1081-6. Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199302
ENTRY DATE: Entered STN: 19930226
Last Updated on STN: 19970203
Entered Medline: 19930205
AB cDNA clones encoding the putative mature forms of the large and small subunits of the potato tuber ADP-glucose pyrophosphorylase have been expressed separately and together in an Escherichia coli B mutant deficient in ADP-glucose pyrophosphorylase activity. Expression of both subunits from compatible vectors resulted in restoration of ADP-glucose pyrophosphorylase activity. Maximal enzyme activity required both subunits. The expressed ADP-glucose pyrophosphorylase was purified and characterized. The recombinant enzyme exhibited catalytic and allosteric kinetic properties very similar to the enzyme purified from potato tuber. The expressed enzyme activity was ***neutralized*** by incubation with antibodies raised against potato tuber and spinach leaf ADP-glucose pyrophosphorylases but not with anti-Escherichia coli enzyme serum.

3-Phosphoglycerate was the most efficient activator and its effect was increased by dithiothreitol. In the ADP-glucose synthesis direction, 3-phosphoglycerate activated the recombinant enzyme nearly 100-fold in the presence of dithiothreitol, with an A0.5 value of 57 microM. The recombinant ADP-glucose pyrophosphorylase was less sensitive to P(i) inhibition and more sensitive to heat denaturation than the potato tuber enzyme. Results suggest that bacterial expression of potato tuber cDNAs could be used to study the role and interaction of the subunits of the native ADP-glucose pyrophosphorylase.

L15 ANSWER 6 OF 8 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1992:190614 HCAPLUS
DOCUMENT NUMBER: 116:190614
TITLE: Chromatographically-purified recombinant human immunodeficiency virus (HIV) glycoprotein gp120 composition retaining natural conformation
INVENTOR(S): Haigwood, Nancy L.; Scandella, Carl J.
PATENT ASSIGNEE(S): Chiron Corp., USA
SOURCE: PCT Int. Appl., 115 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9113906	A1	19910919	WO 1991-US1484	19910308
W: CA, JP, US				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, NL, SE				
CA 2077753	AA	19910910	CA 1991-2077753	19910308
EP 519001	A1	19921223	EP 1991-906615	19910308
EP 519001	B1	20011031		
R: AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE				
JP 05505616	T2	19930819	JP 1991-507168	19910308
JP 09227588	A2	19970902	JP 1996-192595	19910308
AT 207930	E	20011115	AT 1991-906615	19910308
US 5614612	A	19970325	US 1994-240073	19940509
US 5653985	A	19970805	US 1995-439119	19950511
US 5696238	A	19971209	US 1995-439286	19950511
PRIORITY APPLN. INFO.:			US 1990-490858	A2 19900309
			JP 1991-507168	A3 19910308
			WO 1991-US1484	W 19910308
			US 1991-684963	B1 19910820
			US 1993-109002	B1 19930816
			US 1994-240073	A3 19940509

AB Recombinant HIV glycoprotein gp120 is purified in the absence of affinity purifn. steps or any steps using org. solvents by sequentially using (1) ***ion*** ***exchange*** chromatog.; (2) hydrophobic-interaction chromatog.; and (3) size exclusion filtration; and collecting at each step a fraction that exhibits specific binding affinity for CD4 peptide. The product is purified, full-length, nonfusion recombinant HIV gp120 having protein/protein interaction properties identical to native gp120 and is esp. useful for the prodn. of vaccines. The envelope gene encoding gp160 of HIV SF2 variant was engineered for expression of gp120; highest levels of expression in COS-7 cells was seen with cytomegalovirus (CMV) IE-1 promoter. Expression ***plasmid*** pCMV6aSF2-120 was cotransfected with a dhfr expression ***plasmid*** using (Ca)3(PO4)2 copptn. into CHO dhfr-cells to make cell line CHO-A-6a120-145-0.1-22. Recombinant gp120 was purified from the cell culture supernatant by (a) ultrafiltration; (b) DEAE chromatog.; (c) Ph hydrophobic-interaction chromatog.; (d) ether hydrophobic-interaction chromatog.; and (e) gel filtration chromatog. on Superdex 200. A 250-fold purifn. was achieved with a yield of 20-25%. The recombinant gp120 was used to immunize baboons and chimpanzees.

L15 ANSWER 7 OF 8 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1989:226609 HCAPLUS
DOCUMENT NUMBER: 110:226609
TITLE: Manufacture with yeast of immunogenic fragment of plasmodium vivax circumsporozoite protein for use as antimalaria vaccine
INVENTOR(S): Nussenzweig, Victor
PATENT ASSIGNEE(S): New York University, USA
SOURCE: PCT Int. Appl., 40 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 8807546	A1	19881006	WO 1988-US1150	19880330
W: AU, DK, JP, LK				
RW: AT, BE, CH, DE, FR, GB, IT, LU, NL, SE				
AU 8815936	A1	19881102	AU 1988-15936	19880330
AU 617668	B2	19911205		
ZA 8802272	A	19881130	ZA 1988-2272	19880330
EP 309555	A1	19890405	EP 1988-903724	19880330
R: AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE				
ES 2009587	A6	19891001	ES 1988-1013	19880330
JP 01503514	T2	19891130	JP 1988-503280	19880330
DK 8806655	A	19890130	DK 1988-6655	19881129
PRIORITY APPLN. INFO.:			US 1987-32326	19870330
			WO 1988-US1150	19880330

AB A fragment of the P. vivax circumsporozoite (CS) protein which includes the entire tandem repeat sequence plus a region that is conserved in all malaria species is produced in yeast and purified by a simple method which is readily adaptable to scale-up. ***Plasmid*** pAB24 was constructed. It contained a 4.1 kb fragment of the CS protein gene, an alc. dehydrogenase-2-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) hybrid promoter, a GAPDH terminator, and yeast UrA3, Leu2d, and 2 mu sequences. Saccharomyces cerevisiae AB110 was transformed with this ***plasmid*** and cultured to provide 13 mg pure CS fragment/L culture medium. The protein was purified by heat treatment at 100.degree. to ppt. contaminating proteins, and ***ion*** - ***exchange*** and mol. sieve chromatog. of the remaining protein mixt. Monoclonal antibody to P. vivax CS protein reacted with the recombinant CS fragment. Antisera from mice inoculated with this protein ***neutralized*** the infectivity of P. vivax sporozoites in human hepatoma Hep 62 cell culture.

L15 ANSWER 8 OF 8 MEDLINE
ACCESSION NUMBER: 88006398 MEDLINE
DOCUMENT NUMBER: 88006398 PubMed ID: 3308703
TITLE: Identification and characterization of the Pasteurella haemolytica leukotoxin.
AUTHOR: Chang Y F; Young R; Post D; Struck D K
CORPORATE SOURCE: Department of Biochemistry and Biophysics, College of Agriculture, Texas A&M University, College Station 77843.
SOURCE: INFECTION AND IMMUNITY, (1987 Oct) 55 (10) 2348-54.
Journal code: 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198710
ENTRY DATE: Entered STN: 19900305
Last Updated on STN: 19950206
Entered Medline: 19871028

AB The identification and chromatographic characterization of the leukotoxin of Pasteurella haemolytica is described. The toxin, which has an apparent native molecular weight of greater than 400,000 as judged by gel exclusion chromatography, has a 105-kilodalton (105K) polypeptide as its major protein component. The proteolytic degradation of the 105K polypeptide could be correlated with the loss of toxin activity in aging cultures of P. haemolytica. Antisera raised against purified 105K polypeptide ***neutralized*** toxin activity. A 3.9-kilobase-pair fragment of the P. haemolytica genome cloned into a ***plasmid*** vector resulted in the production of intracellular toxin in Escherichia coli host cells. The restriction map of this clone shows significant overlap with the map of a previously reported leukotoxin clone (R. Y. C. Lo, P. E. Shewen, C. A.

Strathdee, and C. N. Greer, Infect. Immun. 50:667-671, 1985). Finally, antisera raised against the 105K species labeled the P. haemolytica cell surface in a nonuniform, punctate manner.

=> d his

(FILE 'HOME' ENTERED AT 09:09:53 ON 18 JUL 2002)

FILE 'BIOSIS, EMBASE, MEDLINE, WPIDS, HCAPLUS' ENTERED AT 09:10:04 ON 18 JUL 2002

L1 334257 S PLASMID?
L2 123300 S LARGE SCALE
L3 1806 S L1 AND L2
L4 297568 S NEUTRALIZ?
L5 1806 S L1 AND L2 AND L3
L6 14 S L1 AND L2 AND L4
L7 10 DUP REM L6 (4 DUPLICATES REMOVED)
L8 2763 S STATIC MIXER
L9 8 S L1 AND L8
L10 7 DUP REM L9 (1 DUPLICATE REMOVED)
L11 297568 S NEUTRALIZ?
L12 244833 S ION(W)(EXCHANGE OR EXCHANGER)
L13 9 S L1 AND L11 AND L12
L14 7 DUP REM L9 (1 DUPLICATE REMOVED)
L15 8 DUP REM L13 (1 DUPLICATE REMOVED)

=> s lysis or lyse or lysing or lysed
L16 144919 LYSIS OR LYSE OR LYSING OR LYSED

=> s l1 and l16

L17 4342 L1 AND L16

=> s l1 and l16 and l2
L18 109 L1 AND L16 AND L2

=> dup rem l18
PROCESSING COMPLETED FOR L18
L19 58 DUP REM L18 (51 DUPLICATES REMOVED)

=> s l19 and py<1997
1 FILES SEARCHED...
3 FILES SEARCHED...
4 FILES SEARCHED...
L20 30 L19 AND PY<1997

=> d l20 ibib abs 1-30

L20 ANSWER 1 OF 30 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1996:508034 BIOSIS
DOCUMENT NUMBER: PREV199699230390
TITLE: A two ***plasmid*** co-expression system in Escherichia coli for the production of virion-like reverse transcriptase of the human immunodeficiency virus type 1.
AUTHOR(S): Jonckheere, Heidi (1); De Vreese, Karen; Debyser, Zeger; Vandekerckhove, Joel; Balzarini, Jan; Desmyter, Jan; De Clercq, Erik; Anne, Jozef
CORPORATE SOURCE: (1) Rega Inst. Med. Research, Katholieke Univ. Leuven, Minderbroedersstraat 10, B-3000 Leuven Belgium
SOURCE: Journal of Virological Methods, (1996) Vol. 61, No. 1-2, pp. 113-125.
ISSN: 0166-0934.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Many bacterial expression systems have been developed to study the reverse transcriptase (RT) of human immunodeficiency virus type 1 (HIV-1). This enzyme exists in the virions as a heterodimer of a 66 kDa (p66) subunit and a 51 kDa (p51) subunit, originating through proteolytic maturation of the p66 subunit. Most expression systems rely on the processing of p66 by bacterial proteases, this results in a p51 subunit with a non-authentic carboxy-terminus. In contrast, the expression system described produces an RT with an authentic carboxy-terminus. This was achieved by the co-expression of the two subunits of HIV-1 RT, which were each cloned on a different, compatible ***plasmid*** in Escherichia coli, and by the use of protease inhibitors during cell ***lysis*** . This approach enabled us not only to obtain virion-like RT, as verified by mass spectrometry, but also to monitor the effect of mutations in one or both subunits on the activity of RT and on its sensitivity towards RT inhibitors. The co-expression system described represents a useful method to produce HIV-1 RT, both authentic and mutated, in quantities that allow ***large*** - ***scale*** studies on the functional organisation of the RT-subunits and the sensitivity of the enzyme to RT inhibitors.

L20 ANSWER 2 OF 30 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1996:483875 BIOSIS
DOCUMENT NUMBER: PREV199699199131
TITLE: Comparison of arbitrarily primed polymerase chain reaction and ribotyping for subtyping Actinobacillus actinomycetemcomitans.
AUTHOR(S): Saarela, Maria (1); Asikainen, Sirkka; Chen, Casey; Alaluusua, Satu; Slots, Jorgen
CORPORATE SOURCE: (1) Inst. Dent., P.O. Box 41, Univ. Helsinki, SF-00014 Helsinki Finland
SOURCE: Anaerobe, (1995) Vol. 1, No. 2, pp. 97-102.
ISSN: 1075-9964.
DOCUMENT TYPE: Article
LANGUAGE: English
AB This study investigated the compatibility of arbitrarily primed polymerase chain reaction (AP-PCR) and ribotyping in the characterization of Actinobacillus actinomycetemcomitans, a major pathogen in the mixed anaerobic microflora of human periodontitis. AP-PCR was performed directly on ***lysed*** bacterial colonies using a random-sequence 10-base oligonucleotide primer. Ribotyping was carried out by using purified bacterial chromosomal DNA digested with BglI. DNA fragments were separated electrophoretically, blotted onto a nylon membrane and hybridized with the ***plasmid*** pKK3535 containing the rRNA operon of Escherichia coli. The two genetic methods were evaluated on isolates from single individuals and from family members. Twelve AP-PCR types and 47 ribotypes were distinguished among 76 A. actinomycetemcomitans isolates of different serotypes. AP-PCR typing and ribotyping gave compatible results in 18 of 20 comparisons. Although AP-PCR detected less genetic heterogeneity in A. actinomycetemcomitans than ribotyping, the rapid and relatively simple AP-PCR technique seems to be sufficiently discriminative to be used in ***large*** ***scale*** epidemiological studies which preclude the application of the more laborious ribotyping technique.

L20 ANSWER 3 OF 30 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1995:252549 BIOSIS
DOCUMENT NUMBER: PREV199598266849
TITLE: ***Large*** - ***scale*** preparation of ***plasmid*** DNA by microwave ***lysis*** .
AUTHOR(S): Wang, Bin; Merva, Mike; Williams, William V.; Weiner, David B. (1)
CORPORATE SOURCE: (1) IBAMM Univ. Pa., 505 BRBI, 422 Curie Drive, Philadelphia, PA 19104 USA
SOURCE: Biotechniques, (1995) Vol. 18, No. 4, pp. 554-555.
ISSN: 0736-6205.
DOCUMENT TYPE: Article
LANGUAGE: English

L20 ANSWER 4 OF 30 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1994:396485 BIOSIS
DOCUMENT NUMBER: PREV199497409485
TITLE: Simplified ***large*** - ***scale*** alkaline ***lysis*** preparation of ***plasmid*** DNA with

minimal use of phenol.
AUTHOR(S): Wang, Lin-Fa (1); Voysey, Rhonda; Yu, Meng
CORPORATE SOURCE: (1) CSIRO Aust. Anim., Health Lab., P.O. Bag 24, Geelong,
Victoria 3220 Australia
SOURCE: Biotechniques, (1994) Vol. 17, No. 1, pp. 26, 28.
ISSN: 0736-6205.
DOCUMENT TYPE: Article
LANGUAGE: English

L20 ANSWER 5 OF 30 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1993:453953 BIOSIS
DOCUMENT NUMBER: PREV199396098853
TITLE: A modified alkaline ***lysis*** method for the preparation of highly purified ***plasmid*** DNA from Escherichia coli.
AUTHOR(S): Feliciello, Isidoro; Chinali, Gianni (1)
CORPORATE SOURCE: (1) CEINGE, Cent. Ingegneria Genetica, Dip. Biochimica
Biotechnol. Med., IIa Fac. Med. Chirurgia, Univ. Napoli, Via Sergio Pansini, 5, I-80131 Naples Italy
SOURCE: Analytical Biochemistry, (1993) Vol. 212, No. 2, pp. 394-401.
ISSN: 0003-2697.
DOCUMENT TYPE: Article
LANGUAGE: English
AB We have developed a very efficient and rapid method for the preparation on
a small or ***large*** ***scale*** of highly purified ***plasmid*** DNA from Escherichia coli. The procedure consists of five
steps: (1) cell ***lysis*** by NaOH-SDS, (2) precipitation of cell lysate with 2 M potassium acetate-1 M acetic acid, (3) precipitation of the resulting supernatant with isopropanol, (4) treatment of the precipitate with RNase, and (5) a second isopropanol precipitation. The new procedure yields a ***plasmid*** DNA that is more than 90% in the
supercoiled form and virtually free from proteins, RNA, and chromosomal DNA. We have thoroughly tested the method in the preparation of several thousand samples of different ***plasmids*** from various E. coli strains. We found that it consistently produced samples of ***plasmid*** DNA suitable for all routine uses such as restriction analysis, sequencing, and preparation of DNA probes for cloning and hybridization experiments. Moreover, ***plasmids*** purified by this procedure could
fully replace ***plasmids*** purified on CsCl gradients for more demanding tasks such as the in vitro synthesis of RNA probes by phage RNA
polymerases, the generation of deletion mutants with exonuclease III, and the transfection of mammalian cells by the calcium phosphate coprecipitation method, as tested on human fibroblasts and on CV-1 cells.

L20 ANSWER 6 OF 30 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1993:453556 BIOSIS
DOCUMENT NUMBER: PREV199396098456
TITLE: Stable expression ***plasmid*** for high-level production of GroE molecular chaperones in ***large*** - ***scale*** cultures.
AUTHOR(S): Kalbach, Cathy E.; Gatenby, Anthony A. (1)
CORPORATE SOURCE: (1) Central Res. Dev., E. I. DuPont de Nemours Co., P.O.
Box 80402, Experimental Station, Wilmington, DE 19880-0402 USA
SOURCE: Enzyme and Microbial Technology, (1993) Vol. 15, No. 9, pp.
730-735.
ISSN: 0141-0229.
DOCUMENT TYPE: Article
LANGUAGE: English
AB A stable expression ***plasmid*** has been developed to overproduces
the Escherichia coli GroES and GroEL molecular chaperones in ***large***
- ***scale*** cultures. This was achieved by cloning the groE operon under the transcriptional control of a bacteriophage T7 promoter to achieve regulated expression. Isopropyl-beta-D-thiogalactopyranoside (IPTG) induction of a lacUV5 regulated chromosomal copy of T7 gene 1, encoding viral RNA polymerase, resulted in high-level expression of the groE operon from a multicopy ***plasmid*** . Induced cells harboring the pT7groE expression ***plasmid*** accumulated GroEL to levels of 30% total cell protein, and GroES to 4-5%. Both overproduced proteins were
recovered primarily from the soluble fraction of ***lysed*** cells. The T7 expression ***plasmid*** was significantly more stable than other groE expression ***plasmids*** tested during scale-up experiments, and could be used successfully for large-volume cultures of up to 200 l. Strain stability was greatly improved, compared to rich media, when cells were grown in a supplemented minimal medium.

L20 ANSWER 7 OF 30 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1993:413277 BIOSIS
DOCUMENT NUMBER: PREV199396079002
TITLE: Isolation and conditioning of recombinant staphylokinase for use in man.
AUTHOR(S): Collen, D. (1); De Mol, M.; Demarsin, E.; De Cock, F.; Stassen, J. M.
CORPORATE SOURCE: (1) Cent. Thrombosis Vascular Res., Univ. Leuven, Campus
Gasthuisberg, O and N, Herestraat 49, B-3000 Leuven Belgium
SOURCE: Fibrinolysis, (1993) Vol. 7, No. 4, pp. 242-247.
ISSN: 0268-9499.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Staphylokinase (STA), a M-r 18 000 protein produced by Staphylococcus
aureus is known to have profibrinolytic properties for more than 40 years (Lack CH, Nature 1948; 161: 559-560) but its potential for thrombolytic therapy has not been adequately investigated. Therefore we have elaborated
procedures for the ***large*** ***scale*** production of recombinant STA (STAR) from the culture broth of E. coli cells transformed
with the recombinant ***plasmid*** pUC19 which contains a 2.9 kb insert obtained by HindIII restriction enzyme digestion of genomic DNA obtained from a selected Staphylococcus aureus strain. STAR, purified from
12 litre batches by chromatography on SP-Sephadex with pH gradient elution, SP-Sephadex with NaCl gradient elution and Sephacryl S-300 superfine gel filtration, with a recovery of 19 +- 4 mg and a yield of 35 +- 15 percent, contained a single band on SDS-polyacrylamide gel electrophoresis with NH-2-terminal sequence
Ser-Ser-Ser-Phe-Asp-Lys-Gly-
Lys-Tyr-Lys-Lys-Gly-Asp-Asp-Ala. It was obtained at a concentration of approximately 1mg/ml with a specific activity of 185 000 +- 35 000 units/mg with an endotoxin content of 10 +- 7 U/mg. After filtration on 0.22 mu-m Millipore filters, the preparations were sterile under aerobic and anaerobic bacterial culture conditions and virus free by routine screening for human pathogenic viruses. The material remained active after
incubation at 37 degree C for several days. Bolus injection of STAR at a dose of 3 mg/kg in mice did not produce weight loss within 8 days. Thus, these materials appear to be suitable for the investigation, on a pilot scale, of the pharmacokinetic and thrombolytic properties of STAR in patients with thromboembolic disease.

L20 ANSWER 8 OF 30 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1992:409659 BIOSIS
DOCUMENT NUMBER: BA94:72859
TITLE: AN IMPROVED METHOD FOR RAPID PURIFICATION OF COVALENTLY
CLOSED CIRCULAR ***PLASMID*** DNA OVER A WIDE SIZE
RANGE.
AUTHOR(S): AZAD A K; COOTE J G; PARTON R
CORPORATE SOURCE: DEP. MICROBIOL., UNIV. GLASGOW, GLASGOW, UK.
SOURCE: LETT APPL MICROBIOL, (1992) 14 (6), 250-254.
CODEN: LAMIE7. ISSN: 0266-8254.
FILE SEGMENT: BA; OLD
LANGUAGE: English
AB An improved method has been developed for the ***large*** -

scale purification of covalently closed circular (CCC) ***plasmid*** DNA molecules of sizes ranging from 4.3 to 73 kb. This protocol uses an alkaline- ***lysis*** procedure followed by acid-phenol extraction but with several modifications to previously reported methods. The principal modification is the replacement of NaCl by MgCl2 in the extraction buffer to improve yield and to remove chromosomal and other non-CCC ***plasmid*** DNA. ***Plasmid*** DNA can be purified in less than 1 h and used successfully in restriction enzyme analysis and cloning experiments.

L20 ANSWER 9 OF 30 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1992:326190 BIOSIS
DOCUMENT NUMBER: BA94:28031
TITLE: ***LARGE*** - ***SCALE*** PURIFICATION OF ***PLASMID*** DNA BY FAST PROTEIN LIQUID CHROMATOGRAPHY USING A HI-LOAD Q SEPHAROSE COLUMN.
AUTHOR(S): CHANDRA G; PATEL P; KOST T A; GRAY J G
CORPORATE SOURCE: MOL. BIOL. DEP., GLAXO INC. RES. INST., FIVE MOORE DRIVE, RESEARCH TRIANGLE PARK, N.C. 27709.
SOURCE: ANAL BIOCHEM, (1992) 203 (1), 169-172. CODEN: ANBCA2. ISSN: 0003-2697.
FILE SEGMENT: BA; OLD
LANGUAGE: English
AB The ***large*** - ***scale*** purification of ***plasmid*** DNA was achieved using fast protein liquid chromatography on a Hi-Load Q Sepharose column. This method allows for the purification of ***plasmids*** starting from crude ***plasmid*** DNA, prepared by a simple alkaline ***lysis*** procedure, to pure DNA in less than 5 h. In contrast to the previously described ***plasmid*** purification methods of CsCl gradient centrifugation or high-pressure liquid chromatography, this method does not require the use of any hazardous or expensive chemicals. More than 100 ***plasmids*** varying in size from 3 to 15 kb have been purified using this procedure. A Mono Q Sepharose column was initially used to purify ***plasmids*** smaller than 8.0 kb; however, a Hi-Load Q Sepharose column proved more effective with ***plasmids*** larger than 8 kb. The loading of ***plasmids*** larger than 8 kb on the Mono Q column resulting in a high back pressure and the ***plasmid*** DNA could not be eluted from the column. Thus, for routine purification we utilize the Hi-Load Q Sepharose column. ***Plasmids*** purified by this method had purity, yield, and transfection efficiency in mammalian cells similar to those of ***plasmids*** purified by CsCl density gradient centrifugation.

L20 ANSWER 10 OF 30 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1991:454376 BIOSIS
DOCUMENT NUMBER: BA92:99156
TITLE: A COMPARATIVE STUDY OF ***PLASMID*** DNA EXTRACTIONS.
AUTHOR(S): HU Y; ET AL
CORPORATE SOURCE: VIRUS RESEARCH INST., HUBEI MED. COLL., WUHAN, CHINA.
SOURCE: ACTA ACAD MED HUBEI, (1991) 12 (2), 104-106. CODEN: HYIXEK.
FILE SEGMENT: BA; OLD
LANGUAGE: Chinese
AB Using agarose gel electrophoresis, we compared three routine DNA extractions of ***plasmids*** through reagents and instruments of our nation. Our results showed that: 1. By ***lysis*** of boiling a lot of plamid DNA could be extracted; 2. ***Lysis*** by alkali damaged CCC conformation of ***plasmid*** DNA by the combination of NaOH and SDS; 3. ***Lysis*** by SDS was suitable for extraction of ***plasmids*** with size of more than 10kbp; 4. ***Lysis*** by boiling is favorable for ***large*** ***scale*** extraction of ***plasmid*** DNA.

L20 ANSWER 11 OF 30 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1989:206953 BIOSIS
DOCUMENT NUMBER: BA87:107857
TITLE: A NEW METHOD OF ***PLASMID*** DNA PREPARATION BY SUCROSE-MEDIATED DETERGENT ***LYSIS*** FROM ESCHERICHIA-COLI GRAM-NEGATIVE AND STAPHYLOCOCCUS-AUREUS GRAM-POSITIVE.
AUTHOR(S): SAHA B; SAHA D; NIYOGI S; BAL M
CORPORATE SOURCE: DEP. OF PHYSIOL., UNIV. COLL. OF SCI. AND TECHNOL., UNIV. OF CALCUTTA, 92 ACHARYA PRAFULLA CHANDRA RD., CALCUTTA-700 009, INDIA.
SOURCE: ANAL BIOCHEM, (1989) 176 (2), 344-349. CODEN: ANBCA2. ISSN: 0003-2697.
FILE SEGMENT: BA; OLD
LANGUAGE: English
AB A simple and cheap method of ***plasmid*** DNA preparation from both gram-positive (Staphylococcus aureus) and gram-negative (Escherichia coli) organism is presented here. In this method, in place of the high-priced chemicals lysostaphin and lysozyme which are commonly used for removal of cell-wall during ***plasmid*** DNA preparation from gram-positive and gram-negative bacteria, respectively, only sucrose has been used. Firstly, bacteria is treated with Trizma (pH 8.0) containing 100% sucrose (hypertonic solution). Due to this osmotic shock, protoplasm covered by the plasma membrane of bacteria possibly shrinks and becomes detached from the cell-wall. Osmotically sensitive cells thus formed, from gram-positive (S. aureus) and gram-negative (E. coli) bacteria, are finally ***lysed*** by the ***lysis*** mixture, containing brij 58 and sodium deoxycholate. The lysate is centrifuged at 15,000 rpm for 30 min to pellet the cell debris. The supernatant containing ***plasmid*** DNA is treated with either polyethylene glycol or isopropanol. The precipitate which contains ***plasmid*** DNA is dissolved in a buffer containing Tris, EDTA, NaCl, and sodium dodecyl sulfate (pH 8.0); thus protein is denatured and removed. Finally, RNA is removed by RNase treatment. The average yield of staphylococcal ***plasmid*** DNA as well as ***plasmid*** pBR322 from E. coli HB101 in 100% sucrose-treated preparations is greater than that of lysostaphin- and lysozyme-treated preparations. This method is applicable for both ***large*** - ***scale*** and small-scale preparations. The substrate activity for restriction enzyme, cloning, transforming ability, and electron microscopic profile of the ***plasmid*** DNA prepared by this method remains unaltered.

L20 ANSWER 12 OF 30 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1989:93213 BIOSIS
DOCUMENT NUMBER: BA87:47349
TITLE: A SIMPLE PROCEDURE FOR ***LARGE*** - ***SCALE*** ***PLASMID*** PREPARATION BY ALKALINE EXTRACTION LITHIUM CHLORIDE PRECIPITATION AND GEL FILTRATION.
AUTHOR(S): PARK J-S
CORPORATE SOURCE: DEP. CHEM., COLL. NATURAL SCI., SEOUL NATL. UNIV.
SOURCE: PROC COLL NAT SCI (SEOUL), (1987) 12 (2), 61-68. CODEN: CKTNDR. ISSN: 0253-6277.
FILE SEGMENT: BA; OLD
LANGUAGE: English
AB A simple inexpensive procedure for pure ***plasmid*** DNA preparation from bacteria is described. Lysozyme-induced spheroplasts are ***lysed*** by a mixture of Brij 58 and sodium deoxycholate. The lysate is centrifugated at 30,000 rpm for 30 min whereby about 99% of total chromosomal DNA is pelleted. The supernatant is titrated up to pH 12.3. Then the pH is adjusted to 9 which facilitates the removal of chromosomal

DNA. Proteins are removed by phenol-chloroform extraction, the high molecular weight RNA by LiCl precipitation, and the small molecular weight RNA by gel filtration on Sepharose CL-2B. The big advantage of the procedure is that its uses no proteinase or RNase to remove proteins or RNA. The whole procedure takes only one day after bacteria are grown. The ***plasmid*** DNA is free from bacterial chromosomal DNA and RNA contamination as assessed by electrophoresis of the preparation on 1% agarose gels and staining with ethidium bromide.

L20 ANSWER 13 OF 30 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1986:377546 BIOSIS
DOCUMENT NUMBER: BA82:72522
TITLE: MODIFIED ***PLASMID*** ISOLATION METHOD FOR CLOSTRIDIUM-PERFRINGENS AND CLOSTRIDIUM-ABSONUM.
AUTHOR(S): ROBERTS I; HOLMES W M; HYLEMON P B
CORPORATE SOURCE: DEPARTMENT MICROBIOLOGY, MEDICAL COLLEGE VIRGINIA, VIRGINIA COMMONWEALTH UNIVERSITY RICHMOND, VA. 23298.
SOURCE: APPL ENVIRON MICROBIOL, (1986) 52 (1), 197-199. CODEN: AEMIDF. ISSN: 0099-2240.
FILE SEGMENT: BA; OLD
LANGUAGE: English
AB A rapid ***plasmid*** isolation procedure for Clostridium perfringens and C. absonum is described. The ratio of culture volume to ***lysis*** buffer volume was found to be crucial for efficent ***plasmid*** isolation. The method can be scaled up, without difficulty, for ***large*** - ***scale*** ***plasmid*** preparation.

L20 ANSWER 14 OF 30 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1984:211647 BIOSIS
DOCUMENT NUMBER: BA77:44631
TITLE: A SIMPLE PROCEDURE FOR ***LARGE*** - ***SCALE*** PREPARATION OF PURE ***PLASMID*** DNA FREE FROM CHROMOSOMAL DNA FROM BACTERIA.
AUTHOR(S): MUKHOPADHYAY M; MANDAL N C
CORPORATE SOURCE: DEP. BIOCHEM., BOSE INST., CALCUTTA-700 009, INDIA.
SOURCE: ANAL BIOCHEM, (1983) 133 (2), 265-270. CODEN: ANBCA2. ISSN: 0003-2697.
FILE SEGMENT: BA; OLD
LANGUAGE: English
AB A very simple, inexpensive procedure for preparing pure ***plasmid*** DNA from bacteria is described. Lysozyme-induced spheroplasts are made in presence of 833 .mu.g/ml of ethidium bromide which are then ***lysed*** by a mixture of Brij 58 and sodium deoxycholate and the lysate is centrifuged at 48,000 g for 25 min, whereby .apprx. 99.9% of total chromosomal DNA is pelleted. From the supernatant ***plasmid*** DNA, the proteins are removed by phenol extraction and the major part of RNA by CaCl2 precipitation and finally the small amount of residual RNA is removed by RNase treatment. The average yield of pBR322 DNA from 1 l of amplified culture by this procedure is 2-2.5 mg and the preparation is highly pure, containing only .apprx. 0.005% of total yield as chromosomal DNA contaminant. The substrate activity and the transforming ability of the ***plasmid*** DNA prepared by this method remain unaffected.

L20 ANSWER 15 OF 30 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1984:185756 BIOSIS
DOCUMENT NUMBER: BA77:18740
TITLE: A METHOD FOR THE PURIFICATION OF ESCHERICHIA-COLI ***PLASMID*** DNA BY HOMOGENOUS ***LYSIS*** AND POLY ETHYLENE GLYCOL PRECIPITATION.
AUTHOR(S): PULLEYBLANK D; MICHALAK M; DAISLEY S L; GLICK R
CORPORATE SOURCE: DEP. BIOCHEM., UNIV. TORONTO, TORONTO, ONT., CAN. M5S 1A8.
SOURCE: MOL BIOL REP, (1983) 9 (3), 191-196. CODEN: MLBRBU. ISSN: 0301-4851.
FILE SEGMENT: BA; OLD
LANGUAGE: English
AB A procedure is described for the isolation and purification of E. coli ***plasmid*** DNA by polyethylene glycol precipitation. The method is rapid, simple, inexpensive and amenable to small and ***large*** ***scale*** manipulation. This procedure involves ***lysis*** of bacterial cells by treatment with pronase in sodium dodecyl sulfate, removal of chromosomal DNA by centrifugation, precipitation of residual nucleic acids with polyethylene glycol and removal of RNA by precipitation with LiCl. ***Plasmid*** DNA purified as described is pure enough for restriction endonuclease analysis, for use as a vector for the cloning of complementary DNA or synthetic DNA, or for use as a template in an E. coli transcription-translation cell-free system.

L20 ANSWER 16 OF 30 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1981:210705 BIOSIS
DOCUMENT NUMBER: BA71:80697
TITLE: RAPID PURIFICATION OF COVALENTLY CLOSED CIRCULAR DNA OF BACTERIAL ***PLASMIDS*** AND ANIMAL TUMOR VIRUSES.
AUTHOR(S): MCMASTER G K; SAMULSKI R J; STEIN J L; STEIN G S
CORPORATE SOURCE: DEP. BIOCHEM. MOL. BIOL., UNIV. FLA., GAINESVILLE, FLA. 32610.
SOURCE: ANAL BIOCHEM, (***1980 (RECD 1981)***) 109 (1), 47-54. CODEN: ANBCA2. ISSN: 0003-2697.
FILE SEGMENT: BA; OLD
LANGUAGE: English
AB A rapid and simple purification of covalently closed circular (supercoiled) DNA from bacterial clones (***plasmids***) and African green monkey cells (SV40-infected) is presented. The method involves immediate treatment of ***lysed*** cells with NaOH, followed by neutralization and phenol extraction in high salt. After the extraction mixture is centrifuged, supercoiled DNA is found in the aqueous phase, the noncovalently closed DNA molecules form a white precipitate at the interphase and proteins pellet. Contaminating RNA is eliminated from the aqueous phase by RNase treatment and precipitation of the supercoiled DNA with polyethylene glycol. Residual polyethylene glycol is removed from the resuspended DNA by chloroform extraction. The purified supercoiled DNA is compatible with restriction enzymes, and is efficient at transforming both .chi. 1776 and HB101 bacterial hosts. Centrifugation in ethidium bromide-cesium chloride or sucrose gradients is not necessary. The method is virtually independent of the molecular size and gives good yields of supercoiled DNA. The technique is applicable to ***large*** - ***scale*** preparations and as a rapid screening procedure in which 20-30 samples can be easily purified within 5-6 h.

L20 ANSWER 17 OF 30 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 94212582 EMBASE
DOCUMENT NUMBER: 1994212582
TITLE: Simplified ***large*** - ***scale*** alkaline ***lysis*** preparation of ***plasmid*** DNA with minimal use of phenol.
AUTHOR: Wang L.-F.; Voysey R.; Yu M.
CORPORATE SOURCE: CSIRO Australian Animal Health Lab., P.O. Bag 24,Geelong,

Vic. 3220, Australia
SOURCE: BioTechniques, (1994) 17/1 (26+28).
ISSN: 0736-6205 CODEN: BTNQDO
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English

L20 ANSWER 18 OF 30 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 77024469 EMBASE
DOCUMENT NUMBER: 1977024469
TITLE: On the isolation of TI ***plasmid*** from Agrobacterium tumefaciens.
AUTHOR: Ledeboer A.M.; Krol A.J.M.; Dons J.J.M.; et al.
CORPORATE SOURCE: Dept. Biochem., State Univ., Leiden, Netherlands
SOURCE: Nucleic Acids Research, (1976) 3/2 (449-463).
CODEN: NARHAD
DOCUMENT TYPE: Journal
FILE SEGMENT: 016 Cancer
029 Clinical Biochemistry
004 Microbiology
LANGUAGE: English
AB An efficient ***lysis*** method for Agrobacterium cells was developed,
which allows a reproducible isolation on the tumor inducing (TI) ***plasmid*** . The ***lysis*** method is based on the sensitivity of this bacterium to incubation with lysozyme, n dodecylamine, EDTA, followed
by Sarkosyl, after growth in the presence of carbenicillin. The authors also present a procedure for the isolation of the TI ***plasmid*** on a ***large*** ***scale*** that might be used for the mass isolation of other large ***plasmids*** which, like the TI ***plasmid*** , cannot be cleared with earlier described procedures. The
purity of the ***plasmid*** preparations was determined with DNA renaturation kinetics, a method that has the advantage that the ***plasmid*** need not to be in the supercoiled or open circular form.

L20 ANSWER 19 OF 30 WPIDS (C) 2002 THOMSON DERWENT
ACCESSION NUMBER: 1997-020828 [02] WPIDS
CROSS REFERENCE: 1996-105920 [11]; 2001-256369 [20]
DOC. NO. CPI: C1997-006674
TITLE: ***Large*** ***scale*** purificn. of ***plasmid*** DNA - by treating microbial cell suspensions by heating and use of an anion exchange matrix and reversed phase HPLC.
DERWENT CLASS: B04 D16
INVENTOR(S): LEE, A L; SAGAR, S
PATENT ASSIGNEE(S): (MERI) MERCK & CO INC
COUNTRY COUNT: 70
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 9636706	A1	19961121	(199702)*	EN	33 <--

RW: AT BE CH DE DK EA ES FI FR GB GR IE IT KE LS LU MC MW NL OA PT SD
SE SZ UG
W: AL AM AU AZ BB BG BR BY CA CN CZ EE GE HU IS JP KG KR KZ LK LR LT
LV MD MG MK MN MX NO NZ PL RO RU SG SI SK TJ TM TR TT UA US UZ VN

AU 9659219	A	19961129	(199712) <--
NO 9705280	A	19980116	(199813)
EP 827536	A1	19980311	(199814) EN

R: AT BE CH DE DK ES FI FR GB GR IE IT LI LU NL PT SE

CZ 9703661	A3	19980415	(199821)
SK 9701557	A3	19980708	(199836)
HU 9802557	A2	19990301	(199916)
JP 11505707	W	19990525	(199931) 32
AU 709003	B	19990819	(199945)
NZ 309231	A	19991028	(199953)
MX 9708967	A1	19980301	(200002)
KR 99014924	A	19990225	(200018)
US 2002001829	A1	20020103	(200207)

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 9636706	A1	WO 1996-US7083	19960515
AU 9659219	A	AU 1996-59219	19960515
NO 9705280	A	WO 1996-US7083	19960515
		NO 1997-5280	19971118
EP 827536	A1	EP 1996-916486	19960515
		WO 1996-US7083	19960515
CZ 9703661	A3	WO 1996-US7083	19960515
		CZ 1997-3661	19960515
SK 9701557	A3	WO 1996-US7083	19960515
		SK 1997-1557	19960515
HU 9802557	A2	WO 1996-US7083	19960515
		HU 1998-2557	19960515
JP 11505707	W	JP 1996-535061	19960515
		WO 1996-US7083	19960515
AU 709003	B	AU 1996-59219	19960515
NZ 309231	A	NZ 1996-309231	19960515
		WO 1996-US7083	19960515
MX 9708967	A1	MX 1997-8967	19971119
KR 99014924	A	WO 1996-US7083	19960515
		KR 1997-708270	19971119
US 2002001829	A1	CIP of US 1994-275571	19940715
		CIP of US 1995-446118	19950519
		Div ex WO 1996-US7083	19960515
		Div ex US 1997-952428	19971107
		US 2001-799906	20010306

FILING DETAILS:

PATENT NO	KIND	PATENT NO
AU 9659219	A Based on	WO 9636706
EP 827536	A1 Based on	WO 9636706
CZ 9703661	A3 Based on	WO 9636706
HU 9802557	A2 Based on	WO 9636706
JP 11505707	W Based on	WO 9636706
AU 709003	B Previous Publ.	AU 9659219
	Based on	WO 9636706
NZ 309231	A Based on	WO 9636706
KR 99014924	A Based on	WO 9636706
US 2002001829	A1 Div ex	US 6197553

PRIORITY APPLN. INFO: US 1995-446118 19950519; US 1994-275571 19940715; US 1997-952428 19971107; US 2001-799906 20010306
AN 1997-020828 [02] WPIDS
CR 1996-105920 [11]; 2001-256369 [20]
AB WO 9636706 A UPAB: 20010515
The following are claimed: (A) a process for ***large*** ***scale*** isolation and purifcn. of ***plasmid*** DNA from ***large*** ***scale*** microbial cell fermentations comprising: (a) harvesting microbial cells from a ***large*** ***scale*** fermentation; (b) adding to the harvested microbial cells a ***lysis*** soln.; (c) heating the microbial cells of (b) to a temp. 70-100 deg. C in a flow through heat exchanger to form a crude lysate; (d) centrifuging the crude lysate; (e) filtering and diafiltering the supernatant of (d) providing a filtrate; (f) contacting the filtrate of (e) with an anion exchange matrix; (g) eluting and collecting ***plasmid*** DNA from the anion exchange matrix; (h) contacting the ***plasmid*** DNA from (g) with a
reversed phase high performance liq. chromatography (RP-HPLC) matrix; (i)
eluting and collecting the ***plasmid*** from the RP-HPLC matrix of (h); (j) optionally concentrating and/or diafiltering the prod. of (i) into a carrier; and (k) optionally sterilising the DNA prod.; and (B) an isolated and purified ***plasmid*** DNA suitable for admin. to humans.
The ***lysis*** soln. is a STET buffer (8% sucrose, 2% Triton (RTM), 50 mM Tris buffer, 50 mM EDTA, pH 8.5).
USE - The method provides for the ***large*** - ***scale*** purifcn. of ***plasmid*** DNA. The prod. can be used in polynucleotide-based vaccines for human use or for human gene therapy.
Dwg.0/9

L20 ANSWER 20 OF 30 WPIDS (C) 2002 THOMSON DERWENT
ACCESSION NUMBER: 1996-105920 [11] WPIDS

CROSS REFERENCE: 1997-020828 [02]; 2001-256369 [20]
DOC. NO. CPI: C1996-033579
TITLE: ***Large*** ***scale*** isolation and purificn. of ***plasmid*** DNA from ***large*** ***scale*** fermentations - involves anion exchange and reversed phase high performance liq. chromatography steps; is suitable for commercial application.
DERWENT CLASS: B04 D16
INVENTOR(S): LEE, A L; SAGAR, S
PATENT ASSIGNEE(S): (MERI) MERCK & CO INC
COUNTRY COUNT: 63
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 9602658	A1	19960201 (199611)*	EN	33	<--

RW: AT BE CH DE DK ES FR GB GR IE IT KE LU MC MW NL OA PT SD SE SZ UG
W: AM AU BB BG BR BY CA CN CZ EE FI GE HU IS JP KG KR KZ LK LR LT LV MD MG MN MX NO NZ PL RO RU SG SI SK TJ TM TT UA US UZ

AU 9531262	A	19960216 (199622)			<--
EP 771355	A1	19970507 (199723)	EN		

R: AT BE CH DE DK ES FR GB GR IE IT LI LU NL PT SE

EP 771355	A4	19970820 (199814)			
JP 10503086	W	19980324 (199822)		31	
AU 708798	B	19990812 (199944)			

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 9602658	A1	WO 1995-US8749	19950711
AU 9531262	A	AU 1995-31262	19950711
EP 771355	A1	EP 1995-927143	19950711
		WO 1995-US8749	19950711
EP 771355	A4	EP 1995-927143	19950711
JP 10503086	W	WO 1995-US8749	19950711
		JP 1996-505121	19950711
AU 708798	B	AU 1995-31262	19950711

FILING DETAILS:

PATENT NO	KIND	PATENT NO
AU 9531262	A Based on	WO 9602658
EP 771355	A1 Based on	WO 9602658
JP 10503086	W Based on	WO 9602658
AU 708798	B Previous Publ.	AU 9531262
	Based on	WO 9602658

PRIORITY APPLN. INFO: US 1994-275571 19940715
AN 1996-105920 [11] WPIDS
CR 1997-020828 [02]; 2001-256369 [20]
AB WO 9602658 A UPAB: 20010515
Prepn. (I) of ***large*** ***scale*** isolation and purificn. of ***plasmid*** DNA from microbial cell fermentations comprises: (a) harvesting the cells, (b) resuspending the cells in ***lysis*** buffer, (c) heating the cells to 70-100 deg. C in a flow-through heat exchanger to form a crude lysate; (d) centrifuging the lysate; (e) filtering and diafiltering the supernatant; (f) contacting the filtrate with an anion exchange matrix; (g) eluting and collecting the ***plasmid*** DNA; (h) purifying the DNA by a reversed phase high performance liq. chromatography step; (i) eluting and collecting the ***plasmids*** ; (j) diafiltering it into a pharmaceutically acceptable carrier, and (k) opt. sterilising the DNA. Also claimed is an isolated and purified ***plasmid*** DNA prepd. as described above.
USE - This is pref. suitable for administering to humans or to non-human animals, and is pref. a polynucleotide vaccine (claimed) or DNA for human gene therapy. (I) can be used to isolate super-coiled, nicked or linearised ***plasmid*** independently.
ADVANTAGE - (I) allows ***large*** ***scale*** commercially viable prepn. of ***plasmid*** DNA, whereas previous methods were suitable only for smaller preparations and were not amenable to scaling up. (I) also removes the need for hazardous and expensive chemicals, e.g. ethidium bromide, is less labour-intensive and time-consuming, results in greater yields and inactivates endogenous DNAses which would degrade the product.
Dwg.0/9

L20 ANSWER 21 OF 30 WPIDS (C) 2002 THOMSON DERWENT
ACCESSION NUMBER: 1995-283770 [37] WPIDS
DOC. NO. CPI: C1995-128074
TITLE: Prodn. of pharmaceutical grade ***plasmid*** DNA - which removes host contaminants and does not rely upon use of toxic organic extractants or mutagenic reagents, e.g. ethidium bromide.
DERWENT CLASS: B04 D16
INVENTOR(S): BUDAHAZI, G; HORN, N; MARQUET, M; MEEK, J
PATENT ASSIGNEE(S): (VICA-N) VICAL INC
COUNTRY COUNT: 20
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 9521250	A2	19950810 (199537)*	EN	36	<--

RW: AT BE CH DE DK ES FR GB GR IE IT LU MC NL PT SE
W: CA JP

WO 9521250	A3	19960215 (199622)			<--
US 5561064	A	19961001 (199645)		17	<--
EP 742820	A1	19961120 (199651)	EN		<--

R: AT BE CH DE DK ES FR GB GR IE IT LI LU MC NL PT SE

JP 09509313	W	19970922 (199748)		50	

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 9521250	A2	WO 1995-US132	19950109
WO 9521250	A3	WO 1995-US132	19950109
US 5561064	A	US 1994-192151	19940201
EP 742820	A1	EP 1995-906763	19950109
		WO 1995-US132	19950109
JP 09509313	W	JP 1995-520613	19950109
		WO 1995-US132	19950109

FILING DETAILS:

PATENT NO	KIND	PATENT NO
EP 742820	A1 Based on	WO 9521250
JP 09509313	W Based on	WO 9521250

PRIORITY APPLN. INFO: US 1994-192151 19940201
AN 1995-283770 [37] WPIDS
AB WO 9521250 A UPAB: 19950921
Prodn. of ***plasmid*** DNA comprises: (i) ***lysing*** cells contg. the ***plasmid*** DNA to obtain a lysate; (ii) treating the lysate by a means for removing insol. material to obtain a solute; and (iii) applying the solute to differential PEG precipitations and chromatography to purify the ***plasmid*** DNA.
USE - The method is used for the prodn. and purificn. of ***plasmid*** DNA that meets all of the standards set by the FDA and other similar organisations, for a pharmaceutical prod. derived from recombinant cells, such as E. coli.
ADVANTAGE - The method is composed of scalable unit operations amenable to ***large*** ***scale*** manufacture. It reliably removes host contaminants such as RNA, host DNA, proteins and lipopolysaccharides and does not rely upon the addn. of extraneous animal-derived proteins such as RNase, lysozyme and Proteinase K. The method does not rely upon the use of toxic organic extractants or mutagenic reagents (e.g. EtBr) and uses only reagents generally recognised as safe by drug regulating bodies such as the FDA.
Dwg.0/1
ABEQ US 5561064 A UPAB: 19961111
A process for purifying ***plasmid*** DNA from host cell impurities (including host chromosomal DNA) to achieve a gene product adapted for clinical use comprising the steps of:
(a) ***lysing*** host cells containing said ***plasmid*** DNA to obtain a lysate and subsequently treating with a salt to precipitate

said host chromosomal DNA;
(b) clarifying said lysate to obtain a clarified lysate;
(c) adding a polyethylene glycol in sufficient quantity to said clarified lysate to obtain a precipitate of said ***plasmid*** DNA;
(d) collecting said precipitate;
(e) dissolving said precipitate to obtain a solution;
(e) adding a salt in sufficient quantity to said solution to precipitate said host cell impurities and to obtain a solute of said ***plasmid*** DNA; and
(f) applying said solute to size exclusion or anion exchange chromatography to obtain said gene product adapted for clinical use;
wherein said process is conducted in the absence of lysozyme, RNase, Proteinase K, phenol, chloroform, and ethidium bromide.
Dwg.0/1

L20 ANSWER 22 OF 30 WPIDS (C) 2002 THOMSON DERWENT
ACCESSION NUMBER: 1987-215081 [31] WPIDS
DOC. NO. CPI: C1987-090247
TITLE: New DNA sequences coding for Streptococcal antitumour protein - and derived expression vectors and transformed E. coli strains.
DERWENT CLASS: B04 D16
INVENTOR(S): AGUI, H; KANAOKA, M; KAWANAKA, C; NEGORO, T
PATENT ASSIGNEE(S): (OHGE-N) OHGEN RES LAB LTD; (ONOG-N) ONOGEN KENKYU-JO KK;
(SUMO) SUMITOMO CHEM IND KK; (SUMU) SUMITOMO PHARM CO LTD
COUNTRY COUNT: 9
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
EP 230777	A	19870805 (198731)*	EN	16	<--
R: CH DE FR GB LI SE					
JP 62158486	A	19870714 (198733)			<--
JP 63052893	A	19880307 (198815)			<--
US 4929547	A	19900529 (199025)			<--
EP 230777	B	19910410 (199115)			<--
R: CH DE FR GB LI SE					
DE 3678702	G	19910516 (199121)			<--
JP 06050989	B2	19940706 (199425)		10	<--
JP 06057153	B2	19940803 (199429)		10	<--

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
EP 230777	A	EP 1986-310075	19861223
JP 62158486	A	JP 1986-194077	19860821
US 4929547	A	US 1986-946025	19861224
JP 06050989	B2	JP 1986-194077	19860821
JP 06057153	B2	JP 1985-298014	19851228

FILING DETAILS:

PATENT NO	KIND	PATENT NO
JP 06050989	B2 Based on	JP 63052893
JP 06057153	B2 Based on	JP 62158486

PRIORITY APPLN. INFO: JP 1985-298014 19851228; JP 1986-194077 19860821
AN 1987-215081 [31] WPIDS
AB EP 230777 A UPAB: 19930922
DNA sequence (I) coding for an antitumour protein (II) of Streptococcus pyogenes is new. Also new are DNA structures (esp. the self-replicating ***plasmid*** pSP1); expression vectors and transformed microorganisms
(esp. E.coli Jm103) contg. (I). (I) contains 2157 bases and codes for a 410 amino acid protein (sequences for both are reproduced in the specification).
S.pyogenes cells are ***lysed*** , chromosomal DNA recovered digested with restriction endonuclease and the fragments fractionated. The 1.8-2.2 kbp fraction was inserted in POC 19 and the modified ***plasmids*** used to transform E.coli JM103. Transformants were tested
for hylridisation with a labelled DNA probe corresponding to a portion of the (II) gene (the N- terminal amino acid sequence of (II) is already partially known). Two clones were positive: they both contained plasmic pSP7 and this was analysed to determine its restriction map. It contains a 2kbp DNA insert at the EcORI site of pUC19. pSP1 was digested with EcORI
and a 210006p fragment, contg. the (II) gene isolated. To produce an expression vector, this fragment was ligated with (a) pKK 223-2 (contg. trp and lac promotors and the gene for ampicillin resistance) to give ptacSP or (b) pIN III Al contg. lpp and lac promoters and the gene for ampicillin resistance) to give pIN III SP; both ***plasmid*** being digested with EcoRI before ligation. The resulting expression ***plasmids*** were inserted into E.coli JM103 to give the new strains ATCC 67271 (pIN III SP) and 67272 (ptac SP). Cultivation of these transformants and induction with isopropyl-beta-D-thiogalactoside resulted
in expression of (II).
USE/ADVANTAGE - (II) can now be synthesised on a ***large*** ***scale*** , without having to culture pathogenic microorganisms, by growing the transformer cells.
0/3
ABEQ EP 230777 B UPAB: 19930922
DNA coding for an antitumour. protein produced by Streptococcus pyogenes
and having the amino acid sequence shown in Figure 2.
ABEQ US 4929547 A UPAB: 19930922
Isolated DNA codes for antitumour protein produced by Streptococcus pyogenes of characteristic amino acid sequence.
Isolated DNA is self-replicating and comprises ***plasmid*** pSP1. Opt. DNA is harboured by expression vector ptacSP or pINIIISP. Host
cell is E. coli JM 103 (pSP1) (ATCC 67270).
ADVANTAGE - Can be purified and analysed by Ouchterlony method or
western blotting method.

L20 ANSWER 23 OF 30 WPIDS (C) 2002 THOMSON DERWENT
ACCESSION NUMBER: 1986-095679 [15] WPIDS
DOC. NO. CPI: C1986-040720
TITLE: Periplasmic mature protein e.g. HGH prodn. - using DNA encoding prokaryotic signals linked to DNA encoding mature eukaryotic proteins.
DERWENT CLASS: B04 D16
INVENTOR(S): BOCHNER, B R; CHANG, C; GRAY, G L; HEYNEKER, H L;
MCFARLAND, N C; OLSON, K C; PAI, R; REY, M W; CHANG, C N;
MCFARIAND, N C; PAI, R C
PATENT ASSIGNEE(S): (GETH) GENENTECH INC
COUNTRY COUNT: 13
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
EP 177343	A	19860409 (198615)*	EN	62	<--
R: AT BE CH DE FR GB IT LI LU NL SE					
JP 61092575	A	19860510 (198625)			<--
US 4680262	A	19870714 (198730)			<--
US 4963495	A	19901016 (199044)			<--
EP 177343	B1	19920722 (199230)	EN	39	<--
R: AT BE CH DE FR GB IT LI LU NL SE					
DE 3586386	G	19920827 (199236)			<--
JP 06296491	A	19941025 (199502)		25	<--
JP 08015440	B2	19960221 (199612)		28	<--
JP 2521413	B2	19960807 (199636)		25	<--

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
JP 61092575	A	JP 1985-222621	19851004
US 4680262	A	US 1984-658339	19841005
US 4963495	A	US 1984-658342	19841005
EP 177343	B1	EP 1985-307044	19851002
DE 3586386	G	DE 1985-3586386	19851002
		EP 1985-307044	19851002
JP 06296491	A Div ex	JP 1985-222621	19851004
		JP 1994-73169	19851004
JP 08015440	B2	JP 1985-222621	19851004

JP 2521413 B2 Div ex JP 1985-222621 19851004
JP 1994-73169 19851004

FILING DETAILS:

PATENT NO	KIND	PATENT NO
DE 3586386	G Based on	EP 177343
JP 08015440	B2 Based on	JP 61092575
JP 2521413	B2 Previous Publ.	JP 06296491

PRIORITY APPLN. INFO: US 1984-658342 19841005; US 1984-658095 19841005; US 1984-658339 19841005
AN 1986-095679 [15] WPIDS
AB EP 177343 A UPAB: 19970502
DNA encoding a prokaryotic secretion signal sequence, pref. an E. coli signal sequence other than that of beta-lactamase, such as an enterotoxin signal sequence, or an AP signal sequence, is operably linked at its 3' end to the 5' end of DNA encoding a mature enkaryotic protein other than chicken triose phosphate isomerase, pref. a mammalian protein such as HGH,
bovine growth hormone or porcine growth hormone.
A method for the periplasmic secretion of a mature eukaryotic protein in the periplasmic space of a host prokaryote comprises (a) constructing a vector for expressing a secretable direct hybrid, which vector contains DNA encoding a prokaryotic secretion signal sequence linked at its 3' end to the 5' end of DNA encoding the mature eukaryotic protein, (b) transforming a prokaryotic host with the vector, (c) culturing the transformed host and (d) allowing mature protein to collect in the periplasm of the host.
USE/ADVANTAGE - The vectors express hybrid preproteins in high yields
in host cells, cleave the signal sequence from the preprotein and secrete mature eukaryotic protein in the periplasmic space of the host cells. The HGH is used for the treatment of hypopituitary dwarfism, burns, wound healinq, dystrophy, bone knitting, diffuse gastric bleeding and pseudoarthrosis.
Dwg.0/6
ABEQ DE 3586386 G UPAB: 19930922
DNA encoding a prokaryotic secretion signal sequence, pref. an E. coli signal sequence other than that of beta-lactamase, such as an enterotoxin signal sequence, or an AP signal sequence, is operably linked at its 3' end to the 5' end of DNA encoding a mature enkaryotic protein other than chicken triose phosphate isomerase, pref. a mammalian protein such as HGH,
bovine growth hormone or porcine growth hormone.
A method for the periplasmic secretion of a mature eukaryotic protein in the periplasmic space of a host prokaryote comprises (a) constructing a vector for expressing a secretable direct hybrid, which vector contains DNA encoding a prokaryotic secretion signal sequence linked at its 3' end to the 5' end of DNA encoding the mature eukaryotic protein, (b) transforming a prokaryotic host with the vector, (c) culturing the transformed host and (d) allowing mature protein to collect in the periplasm of the host.
USE/ADVANTAGE - The vectors express hybrid preproteins in high yields
in host cells, cleave the signal sequence from the preprotein and secrete mature eukaryotic protein in the periplasmic space of the host cells. The HGH is used for the treatment of hypopituitary dwarfism, burns, wound healinq, dystrophy, bone knitting, diffuse gastric bleeding and pseudoarthrosis.
ABEQ EP 177343 B UPAB: 19930922
A hybrid DNA sequence encoding a protein having at least the amino terminal sequence of mature hGH operably linked to a DNA sequence encoding
an enterotoxin signal.
0/0
ABEQ US 4680262 A UPAB: 19930922
Protein (PR) is recovered from the periplasmic space of a bacterial cell transformed to secrete an eukaryotic PR by (A) contacting the cell with sufficient of a 2-4C alkanol, pref. EtOH or butanol, for a sufficient time to kill the cell without ***lysing*** the inner membrane, (B) freezing the cell and then thawing the cell and (C) recovering the periplasmic PR including the eukaryotic PR from the cell.
The cell is pref. heated to 35-55 deg.C for 0.5-20 mins., with the heating and contacting carried out simultaneously while the cell in an aq. suspension in the culture medium. The alkanol concn. is 0.5-10, esp. 1.5, vol.%. The suspension of thawed cell is diluted into a tris buffer. The PR is a mature eukaryotic PR. The cell is esp. E. coli and the PR is human growth hormone.
ADVANTAGE - Proteolytic degradation by proteases during recovery is
minimised as is contamination of the periplasmic PR by intracellular PR; a more viable, ***large*** ***scale*** process than known ones; use of contaminating proteinaceous reagents is avoided.
ABEQ US 4963495 A UPAB: 19930922
Recombinant DNA sequences that encode the formation of mature human growth
hormone are operably linked at the DNA region which encodes the terminal
amine gp. to a DNA sequence that encodes the STII signal.
Plasmids
for the transformation of suitable microorganisms have been isolated, e.g. pAP-STII-hGH, ptrp-STII-hGH and pAP-1.
USE - Escherichia coli are transformed and then selectively propagated to produce mature human growth hormone as a heterologous protein.

L20 ANSWER 24 OF 30 WPIDS (C) 2002 THOMSON DERWENT
ACCESSION NUMBER: 1986-001203 [01] WPIDS
DOC. NO. CPI: C1986-000437
TITLE: Hybrid ***plasmid*** of tryptophan promoter and beta-galactosidase gene - useful in host microorganism for ***large*** ***scale*** prodn. of beta-galactosidase.
DERWENT CLASS: B04 D16
INVENTOR(S): BEPPU, T; KURIHARA, T; MASUDA, K; ODAWARA, Y; SHIMIZU, N
PATENT ASSIGNEE(S): (HITA) HITACHI LTD
COUNTRY COUNT: 4
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
EP 165614	A	19851227	(198601)*	EN	18 <--
R: DE FR GB					
JP 61009287	A	19860116	(198609)		<--
JP 04006353	B	19920205	(199209)		<--

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
EP 165614	A	EP 1985-107679	19850621
JP 61009287	A	JP 1984-127471	19840622
JP 04006353	B	JP 1984-127471	19840622

PRIORITY APPLN. INFO: JP 1984-127471 19840622
AN 1986-001203 [01] WPIDS
AB EP 165614 A UPAB: 19930922
(1) Hybrid ***plasmid*** comprising a tryptophan promoter and beta-galactosidase gene connected to a DNA coding for 8 amino acids at a N-terminal side of tryptophan E polypeptide on the downstream side of the
tryptophan promoter. (2) Hybrid ***plasmid*** pTREZ 1 as characterised
by a restriction map and by a base sequence is new. (3) Prepn. of hybrid ***plasmid*** pTREZ 1 comprises (a) digesting ***plasmid*** pTRE 1
with EcoRI and Sal I to obtain a DNA fragment contg. a tryptophan promoter
(I); (b) purifying the DNA fragment contg. (I) by agarose gel electrophoresis and (c) ligating DNA fragment (I) and beta-galactosidase gene with T4 DNA ligase. (3) Micro-organism contg. or harbouring a hybrid
plasmid as defined in paragraph (1) above is new. (4) Escherichia
coli strains M182 and HB101 and harbouring ***plasmid*** pTREZ 1 and
deposited respectively as FERM BP-816 and BP-815 are new. (5) Prodn. of
beta-galactosidase (II) comprises (a) cultivating E. coli harbouring a hybrid ***plasmid*** in a culture medium; (b) adding an inducer during the cultivation to induce (II) prodn.; (c) recovering the E. coli; (d) ***lysing*** the E. coli and crushing the cells; (e) extrng. (II) out of

the cell mixt., and (f) purifying the extra. (II).
USE/ADVANTAGE - The hybrid ***plasmid*** permits prod. of (II) on
a ***large*** ***scale*** . The ***plasmid*** may be used for searching for new promoters or for detecting the prodn. of foreign genes by expression of (II). See E.P. 165613. (II) is useful for modifying milk for patients suffering from alactosia.
0/3

L20 ANSWER 25 OF 30 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1995:610625 HCAPLUS
DOCUMENT NUMBER: 123:8040
TITLE: Extraction of polypeptide inclusion bodies from expression hosts with a two-phase aqueous system with solubilization and renaturation of the polypeptide
INVENTOR(S): Builder, Stuart; Hart, Roger; Lester, Philip; Ogez, John; Reifsnyder, David
PATENT ASSIGNEE(S): Genentech, Inc., USA
SOURCE: PCT Int. Appl., 69 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9506059	A1	19950302	WO 1994-US9089	19940810 <--
W: AU, CA, JP, US				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
US 5407810	A	19950418	US 1993-110663	19930820 <--
CA 2167910	AA	19950302	CA 1994-2167910	19940810 <--
AU 9475616	A1	19950321	AU 1994-75616	19940810 <--
AU 673624	B2	19961114		
EP 714403	A1	19960605	EP 1994-925830	19940810 <--
EP 714403	B1	19980610		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE				
JP 09501931	T2	19970225	JP 1994-507623	19940810
AT 167193	E	19980615	AT 1994-925830	19940810
ES 2119222	T3	19981001	ES 1994-925830	19940810
US 5723310	A	19980303	US 1995-385187	19950207
US 5695958	A	19971209	US 1995-446882	19950517

PRIORITY APPLN. INFO.: US 1993-110663 19930820
WO 1994-US9089 19940810
US 1994-318627 19941011
US 1995-385187 19950207

AB A method is described for isolating an exogenous polypeptide in a non-native conformation from cells, such as an aq. fermn. broth. The inclusion bodies are incubated in a soln. of a chaotropic agent contg., preferably, a reducing agent and with phase-forming species to form multiple aq. phases, with one of the phases being enriched in the polypeptide and depleted in the biomass solids and nucleic acids originating from the cells. The method results in two aq. phases, with the upper phase being enriched in the polypeptide. A ***large*** ***scale*** (1200 L) fermn. of Escherichia coli accumulating inclusion bodies of insulin-like growth factor 1 as a result of expression of the cloned gene was ***lysed*** with urea 174 kg and dithiothreitol 2.9 kg and brought to pH 10 with NaOH. The lysate was mixed with PEG-8000 250
and sodium sulfate 90 kg and the phases allowed to sep. The upper phase contained 88% of the total IGF-1 in the prepn. The upper phase was collected and neutralized to ppt. the IGF-1 and the pptd. material was resuspended in a folding medium of urea 10, NaCl 1 M, EtOH 19 vol%, glycine 20 mM, copper 0.5 .mu.M, DTT 1mM pH 10.5. Renaturation had reached a plateau at 3 h with a 50% yield of folded IGF-1.

L20 ANSWER 26 OF 30 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1994:189732 HCAPLUS
DOCUMENT NUMBER: 120:189732
TITLE: Manufacture of particles containing hepatitis B virus surface (preS1+preS2+S) and core antigens
INVENTOR(S): Kniskern, Peter J.; Hagopian, Arpi; Burke, Pamela
PATENT ASSIGNEE(S): Merck and Co., Inc., USA
SOURCE: PCT Int. Appl., 82 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9401132	A1	19940120	WO 1993-US6252	19930630 <--
W: AU, BB, BG, BR, CA, CZ, FI, HU, JP, KR, KZ, LK, MG, MN, MW, NO, NZ, PL, RO, RU, SD, SK, UA, US				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
AU 9346599	A1	19940131	AU 1993-46599	19930630 <--

PRIORITY APPLN. INFO.: US 1992-910221 19920707
WO 1993-US6252 19930630

AB Hepatitis B virus (HBV) surface and core protein antigens are manufd. as
mixed particles by expression of genes for the antigens in a single host yeast. To form particles with substantially reduced carbohydrate, the genes are expressed in a glycosidation-deficient host. These mixed particles display, on the same particle, antigenic sites of the envelope domains (including preS and S) and the core antigen and also have a low nucleic acid content. These particles are useful in vaccines for active and passive treatment or prevention of HBV disease and infection and serol. related agents including surface protein antigenic variants (esp. in populations hypo- or non-responsive to other HBV vaccines), and also as
reagents for use in diagnostic tests. The genes for the core antigens of adw and ayw serotypes were placed under control of the GAP promoter and
the gene for the preS1+preS2+S antigen was placed under control of the GAL10 promoter. Potential glycosidation sites were removed by site-directed mutagenesis. A bidirectional expression construct for the two antigen genes using the GAL1/10 promoter was also prepd. The nucleic
acid-binding domain of the core antigen was removed by deletion of the coding region for the C-terminal Arg-rich region. Mnn9-glycosidation-deficient Saccharomyces cerevisiae hosts were prepd. and transformed with the expression constructs. Transformants were ***lysed*** and shown to contain 28 nm particles that contained core and
surface antigens. ***Large*** ***scale*** manuf. of the particles is described.

L20 ANSWER 27 OF 30 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1990:194661 HCAPLUS
DOCUMENT NUMBER: 112:194661
TITLE: A simple single-step procedure for small-scale preparation of Escherichia coli ***plasmids***
AUTHOR(S): He, M.; Wilde, A.; Kaderbhai, M. A.
CORPORATE SOURCE: Dep. Biochem., Univ. Coll. Wales, Aberystwyth, SY23 3DD, UK
SOURCE: Nucleic Acids Res. (***1990***), 18(6), 1660
CODEN: NARHAD; ISSN: 0305-1048
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Numerous rapid procedures to prep. relatively pure ***plasmid*** DNA
from small vols. of E. coli cultures have been developed for restriction mapping purposes. Some of these procedures demand lengthy fractionation
steps involving lysozyme-mediated cell ***lysis*** , boiling, phenol/chloroform extns. and DNA pptns. A procedure which allows isolation of ***plasmid*** DNA on a miniscale, demanding no more than
20 min, is reported here. The principle is based on the finding that phenol/chloroform treatment of E. coli cells in the presence of LiCl and Triton X-100 solubilizes the ***plasmid*** DNA, concomitantly pptg. the unwanted denatured chromosomal DNA and the cellular proteins. The procedure also works well for ***large*** - ***scale*** prepn. (from 5 to 50 mL cultures) of ***plasmids*** . The ***plasmid*** fractionated immediately after TELT/phenol/chloroform was also suitable for direct transformation of competent E. coli cells. That such ***plasmids*** introduced into E. coli were intact and functional was confirmed by the ability of (i) pBR322 to impart the bacterium with resistance to ampicillin and tetracycline, and (ii) the construct pEI-W3

to express upon thermo-induction the cloned heterologous gene product, pre-SS-RUBISCO.

L20 ANSWER 28 OF 30 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1988:623899 HCAPLUS
DOCUMENT NUMBER: 109:223899
TITLE: Construction of a Bacillus-E. coli shuttle vector
AUTHOR(S): Liu, Wen Hsiung; Wang, Yuh Hwa
CORPORATE SOURCE: Dep. Agric. Chem., Natl. Taiwan Univ., Taipei, Taiwan
SOURCE: Chung-kuo Nung Yeh Hua Hsueh Hui Chih (***1988***), 26(2), 173-8
CODEN: CKNHAA; ISSN: 0578-1736
DOCUMENT TYPE: Journal
LANGUAGE: Chinese
AB The Bacillus ***plasmid*** pHW1 (CmR EMR, 4334 bp) and the Escherichia coli ***plasmid*** pBR322 (ApR TcR, 4362 bp) were isolated from B. subtilis MI-112 and E. coli HB 101, resp., and cleavaged by restriction endonuclease HindIII. The two linear ***plasmid*** were mixed and ligated as a hybrid ***plasmid*** by T4 DNA ligase. After transformation of this hybrid ***plasmid*** to E. coli C-600, it was found that ApRCmREmRTcS transformants could be isolated. The expression of CmR and EmR characteristics in E. coli transformants indicated that the antibiotic resistant gene of Bacillus ***plasmid*** pHW1 could be expressed in E. coli host system. The hybrid ***plasmid*** (pHW1-pBR322) could be isolated from ***large*** - ***scale*** culture of ApRCmREmRTcS transformants by alkali ***lysis*** method.
According to the results of HindIII cleavaged and agarose gel electrophoresis analyses, it was confirmed that the hybrid ***plasmids*** were covalently closed circular hybrid ***plasmid*** (8.7 kb) and could be used as a Bacillus-E. coli shuttle vector.

L20 ANSWER 29 OF 30 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1983:607386 HCAPLUS
DOCUMENT NUMBER: 99:207386
TITLE: Alpha-interferon Gx-1
INVENTOR(S): Sloma, Alan
PATENT ASSIGNEE(S): Bristol-Myers Co. , USA
SOURCE: Eur. Pat. Appl., 41 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 89692	A2	19830928	EP 1983-102893	19830323 <--
EP 89692	A3	19840328		
EP 89692	B1	19900228		
R: AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE				
CA 1210714	A1	19860902	CA 1983-422646	19830301 <--
JP 58201798	A2	19831124	JP 1983-47349	19830323 <--
JP 08022230	B4	19960306		
AT 50597	E	19900315	AT 1983-102893	19830323 <--
US 4695543	A	19870922	US 1984-602275	19840424 <--
US 4748233	A	19880531	US 1987-55044	19870528 <--

PRIORITY APPLN. INFO.: US 1982-361364 19820323
EP 1983-102893 19830323
US 1984-602275 19840424

AB Gene Gx-1, which codes for human .alpha.-interferon (IFN-.alpha.), is cloned in Escherichia coli. Thus, the IFN-.alpha. mRNA extd. from Newcastle disease virus-treated human leukocytes was used as a template to prep. cDNA. This cDNA was inserted into ***plasmid*** pBR322 by the GC-tailing technique, and the recombinant ***plasmid*** DNA was transformed into E. coli HB101. Transformants were screened for the presence of the Gx-1 gene by hybridization with a synthetic oligonucleotide probe having a sequence homologous to portions of several human IFN genes. From 1 clone (A3-26), the Gx-1 gene was excised and then characterized by restriction mapping and sequence anal. The nucleotide and encoded peptide sequences of this gene were detd. Substantially pure human IFN-.alpha. was obtained by culturing A3-26 cells in ***large*** ***scale*** , followed by ***lysis*** of the cells and extn. and purifn. of IFN-.alpha. by known techniques.

L20 ANSWER 30 OF 30 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1980:142823 HCAPLUS
DOCUMENT NUMBER: 92:142823
TITLE: ***Large*** - ***scale*** purification of two forms of active lac operator from ***plasmids***
AUTHOR(S): Kallai, Olga B.; Rosenberg, John M.; Kopka, Mary L.; Takano, Tsunehiro; Dickerson, Richard E.; Kan, James; Riggs, Arthur D.
CORPORATE SOURCE: Div. Chem. Chem. Eng., California Inst. Technol., Pasadena, CA, 91125, USA
SOURCE: Biochim. Biophys. Acta (***1980***), 606(1), 113-24
CODEN: BBACAQ; ISSN: 0006-3002
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A procedure is described for obtaining milligram quantities of a small (29 nucleotide) Eco RI restriction fragment of DNA contg. the Escherichia coli lac operator. The purifn. comprises ***lysis*** of cells and removal of bulk chromosomal DNA, digestion and removal of bulk RNA and protein, phenol extn., agarose gel filtration, restriction enzyme cleavage of operator from ***plasmid*** DNA and its sepn. from the cut ***plasmid*** on agarose. Final purifn. is by gel filtration lyophilization and desalting. A yield of 10-15 mg of operator is obtained from 1 kg of wet cell paste. The resultant operator is homogeneous and competitively active in filter assays. Two separable but interconvertible forms of lac operator exist in soln., probably linear duplex and hairpin isomers. Only the presumed linear form is active in binding lac repressor by competition assay, but the 2 isomers are interconvertible by heating to 80.degree.. The methods described here should be generally applicable for purifying other restriction fragments from ***plasmids*** .